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Cytogenetic and Genome Research

38th Biennial American Cytogenetics Conference

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Skamania Lodge
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2004 American Cytogenetics Conference Distinguished Cytogeneticist Award to R. Ellen Magenis, MD

The physician-cytogeneticist behind the disorder Smith-Magenis syndrome, Dr. Ellen Magenis has contributed significantly to the field of cytogenetics over the last 35 years. From her early days of gene mapping to clinical diagnosis to microdeletion syndrome delineation, Dr. Magenis has taken advantage of each technological breakthrough, combined with her clinical acumen, to stay on the cutting edge of cytogenetic research and clinical testing.

Born in Gary, Indiana in 1925, Dr. Magenis received her BA in zoology from Indiana University in 1946 and her MD from Indiana University Medical School in 1952. While serving in the role of devoted and nurturing mother to her six children, Dr. Magenis completed a residency program in pediatrics at the University of Oregon Medical School (now Oregon Health and Science University) and then joined Dr. Fred Hecht for a 3-year fellowship in medical genetics. She became an instructor in the Department of Medical Genetics and the Crippled Children's Division and quickly rose in rank to full professor in 1980. She is board certified in pediatrics by the American Board of Pediatrics, and in clinical cytogenetics and clinical genetics by the American Board of Medical Genetics. A founding fellow of the American College of Medical Genetics, she has served on many editorial boards and has published over 150 papers, 120 abstracts and 20 book chapters. She has served as teacher and mentor to numerous medical students, graduate students, residents and fellows. On the regional level, Dr. Magenis served as Director of the Pacific Northwest Regional Genetics Group Cytogenetics Quality Assurance Committee. Nationally, she has been a member of the Southwest Oncology Group since 1985, serving as Chairman of the Cytogenetics Committee for eleven years, and filled the role of Chairman of the Germ Cell Tumor Cytogenetics Subcommittee, Children's Cancer Group. Her devotion as advocate for patients and their families is evident locally and nationally by her efforts on the board of PRISMS (National Smith-Magenis Syndrome Association), the Angelman Syndrome Association, the Prader-Willi Syndrome Association, the Multnomah County Prader-Willi Project Advisory Group and the Prader-Willi Parent Support Group of Oregon.

Dr. Magenis is currently contributing her expertise fulltime at Oregon Health and Science University as Professor of Molecular and Medical Genetics, Pediatrics and Child Development and Rehabilitation Center (CDRC), Medical Director of Cytogenetics for the Department of Molecular and Medical Genetics, Director, Molecular Cytogenetics Research Laboratory and Training Coordinator for Genetics, CDRC. Dr. Magenis is Director of the Chromosome Clinic and a faculty geneticist for the Genetic and Birth Defects Clinic, CDRC, where she is a fully active clinician, continuing her tender patient care.

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ACC 2006 and Wrap-up
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1

The DNA-Based Structure of Human Chromosomes in Interphase Nuclei

Uwe Claussen

Institute of Human Genetics and Anthropology,
Friedrich Schiller University, Jena, Germany

In contrast to metaphase chromosomes, little is known about the shape, length and architecture of human interphase chromosomes mainly due to technical problems in visualizing interphase chromosomes in total. We analyzed the structure of chromosomes in interphase nuclei using high-resolution multicolor banding (MCB), which paints the total shape of chromosomes and creates a DNA-mediated, chromosome region-specific, pseudocolored banding pattern at high resolution. Furthermore, chromosome stretching experiments were performed and the process of metaphase spreading on the slide was investigated in detail. The results show that the shape and banding pattern of interphase chromosomes of lymphocytes and HeLa cells are similar to those of the corresponding metaphase chromosomes at all stages of the cell cycle. In lymphocytes, the length of the axis of interphase chromosomes is comparable to that of a metaphase chromosome at 600-band resolution. The MCB pattern also allows the detection and characterization of chromosome aberrations. This may be of fundamental importance in establishing chromosome analyses in non-dividing cells. Chromosome preparation is composed of a dramatic water induced swelling of the mitoses taking place during evaporation of the fixative on the slide. The swelling of the mitoses which is responsible for chromosome stretching, the appearance of the G-banding pattern, and metaphase spreading is based on an interaction of acetic acid, water and cellular proteins. Chromosomes have their own potential to swell and to get longer during chromosome preparation. Consequently, we strongly recommend to reassess the concept of chromosome condensation during mitosis and to replace it by the new concept of chromosome region specific protein swelling.

Travel in part sponsored by Chroma Technology Corporation.

2

A View of Early Cytogenetics in the Pacific Northwest (1959–1963)

Jean H. Priest

Professor Emeritus, Pediatrics – Medical Genetics, Emory University, Atlanta, GA, USA

When I arrived at the Rainier School for handicappepd children, located in Buckley, WA, as staff Pediatrician in 1958, I found an environment providing unbelievable challenge for both laboratory and clinical geneticists (if we could be called that). I 'moon-lighted' at the University of Washington in the Departments of Medicine (Arno Motulsky) and Genetics (Stan Gartler), invaluable mentors. At Rainier School, Darwin Norby and Horace Thuline also worked to set up laboratory facilities. Jerry LaVeck as hospital director gave full support. There were over 100 institutionalized cases with clinical Down syndrome (DS); two large dormatories housed undiagnosed severe mental retardation; a subacute hospital included cases of hydrocephaly, anencephaly and other severe birth defects admitted for custodial care; epidemics of infectious disease had to be controlled. Where to start?

We gave priority to cytogenetics. Three years before, Tjio and Levan had determined the correct count for humans, and the extra G group chromosome in DS had just been described as well as triple X. Critical laboratory steps early in this journey of discovery were hypotonic treatment prior to fixation and later (after we had started), use of mitogen (phytohemagglutinin, PHA) to stimulate peripheral blood lymphocytes. Our cytogenetics began in a broom closet and expanded to a small ward kitchen service area. The first chromosome studies were on bone marrow using direct squash technique with aceto orcein causing permanently stained red thumb! Then PHA came along and things got easier but were unreliable because we made our own PHA from red kidney beans processed in a blender.

Chromosome studies in this early period included: 1) Werner syndrome – no abnormality [Lancet]; 2) a trisomic DS mother, her non-DS twins and her family, with assessment of risk for abnormal segregation [Amer J Dis Child]; 3) G trisomy and a de novo translocation DS in a family [Lancet]; 4) XXY chromosome constitution in a male callico cat [Science].

Current techniques in molecular cytogenetics make these early studies seem crude. Nevertheless, they established a number of basic principals on which further knowledge could be built. With the field of discovery wide open it was an era never to be duplicated.

3

Prenatal Diagnosis of 28 de novo Supernumerary Marker Chromosomes not Deriving from Chromosome 15

B. Huang[a, b], S. Ungerleider[c], M. Thangavelu[a, b]

[a] Genzyme Genetics, Orange, CA, [b] University of California, Irvine, CA, [c] Kaiser-Permanente Medical Group of Northern California, USA

The prevalence of supernumerary marker chromosomes (SMC) has been estimated to be 0.6–1.5 per thousand in prenatal studies. With the exception of inv dup(15), SMCs pose a challenging counseling situation, as it is difficult to predict the phenotypes. In an attempt to evaluate the pregnancy outcome of the prenatally-diagnosed marker chromosomes, we reviewed the clinical and cytogenetic data of the prenatally diagnosed SMCs other than those derived from chromosome 15. To date, clinical information regarding pregnancy outcome was obtained for a total of 28 de novo SMC cases. Among these cases, 22 had at least one SMC originated from non-acrocentric chromosomes including chromosomes 4, 5, 6, 7, 8, 10, 11, 12, 16, 17, 18, 19, 20 and yet to be identified chromosomes. The remaining were originated from acrocentric chromosomes other than chromosome 15. Clinical follow-up obtained in these cases ranged from birth to 20 months of age. All 6 cases with acrocentric SMCs resulted in normal phenotypes, while 9/22 non-acrocentric SMCs resulted in abnormal phenotype. Fetal abnormalities were detected by prenatal ultrasound examination in 7 of the 9 cases with abnormal phenotypes. In summary, our results are consistent with the previous reports indicating an overall low risk for acrocentric SMCs and higher risk for non-acrocentric SMCs. In addition, our study shows that prenatal ultrasound examinations may be helpful in detecting abnormalities associated with SMCs.

4
Diploid/Triploid Mixoploidy with 46,XX/69,XXY

L.M. Pasztor[a, b], J. Skierkowski[b], M.A. Pearson[c]

[a] Clin-Path Associates, [b] Palo Verde Laboratory, a Division of Sonora Quest Laboratories, Tempe, AZ, [c] Neonatology Associates, Phoenix, AZ, USA

In contrast to true triploidy, diploid/triploid mixoploidy is a rare and less severe condition in which infants can survive beyond the neonatal period. In addition to the normal cell line, a second triploid cell line is present in varying degrees and with diverse tissue distribution. The blood karyotype is normal in 75% of published cases; the correct diagnosis has been made only after chromosome analyses of other tissues such as skin fibroblasts.

We present a child with 46,XX/69,XXY mixoploidy, a rare chromosome complement among published cases of diploid/triploid mosaicism.

The child was born after in vitro fertilization at 38 weeks gestation via induced vaginal delivery due to oligohydramnios to a then 35-year-old gravida 3, para 2, TAB1 mother and 37-year-old father. There was reduced fetal movement as well as prenatal growth retardation. When examined at 3 year, 2 months, her early development appeared markedly delayed. Physical examination showed several minor anomalies including syndactyly, clinodactyly, abnormal palmar creasing and skin pigmentary anomalies. A standard peripheral blood chromosome study showed a 46,XX chromosome complement. Differential diagnoses included Russell-Silver syndrome, Hypomelanosis of Ito and mosaic chromosome complement in a non-hematopoietic tissue.

Four years later the child was referred because of recurrent fractures. She was petite, but proportionate, normocephalic with normal female external genitalia. Interestingly, developmental assessment revealed only mild delay. Several areas of hyper- and hypopigmented skin were pronounced. Cytogenetic analysis of cells from skin fibroblast cultures showed a 69,XXY[31]/46,XX[39] chromosome complement. Interphase FISH studies with X centromere and SRY probes revealed that 29/143 nuclei contained two X chromosomes and were also SRY+. However, each of 500 nuclei from peripheral blood contained two X chromosomes with no evidence for SRY. Collagen analyses of skin fibroblasts showed normal results.

Diploid/triploid mixoploidy is likely an underdiagnosed condition. Chromosome analysis of fibroblast cells should be considered in patients with growth retardation and syndactyly, especially in those showing skin pigmentation abnormalities. Differential diagnoses for several patients with confirmed diploid/triploid mixoploidy have included Russell-Silver syndrome as well as Hypomelanosis of Ito.

5
Microduplication of Chromosome 17 Involving the Miller-Dieker Region; dup(17)(p13.2p13.3)

Hutton M. Kearney[a], Kathleen Kaiser-Rogers[b, c], Kailas N. Nandi[a], Chrystal W. Murphy[a], Arthur S. Aylsworth[b, c], Kathleen W. Rao[a, b, c]

[a] Department of Pathology and Laboratory Medicine, [b] Department of Pediatrics, Division of Genetics and Metabolism, [c] Department of Genetics, University of North Carolina, Chapel Hill, NC, USA

Complementary microduplications have already been reported for several microdeletion syndromes including Prader-Willi/Angelman syndromes (15q11-q13), Smith-Magenis syndrome (17p11.2), and DiGeorge/VCS syndrome (22q11.2). There have been, however, no reports to date of duplications complementary to the 17p13.3 deletions associated with Miller-Dieker syndrome. We have studied a 3½-year-old individual with a small interstitial duplication of chromosome 17 that encompasses this region. The duplication was visible at the 800 band level and verified by FISH studies. Specifically, we observed a duplicated signal with a probe for the LIS1 locus and a single signal with a 17p subtelomeric probe. Parental studies were normal, indicating that the duplication represents a new mutation in this family. As observed for other complementary microduplication syndromes, the clinical findings in this child were milder than those typically seen in the corresponding microdeletion syndrome. This child has a history of hypotonia in early infancy and significant speech delay (only babbles at 3½ years). Her behavior problems include extreme touch avoidance and tantrums. Growth is normal and no striking dysmorphic features were noted. She has a round face and the suggestion of slight telecanthus and/or slightly short palpebral fissures which could not be measured due to her behavior.

6
Haploinsufficiency for DNA Damage Response Genes in Tumor Cells with 11q13 Amplification

S.M. Gollin, R.A. Parikh, X. Huang, R. Baskaran

Departments of Human Genetics and Molecular Genetics & Biochemistry, University of Pittsburgh and the University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

Many of the 'hallmarks of cancer', including defects in genome stability, are interconnected through the DNA damage response, which involves sensing of DNA damage followed by transduction of the damage signal to a complex network of cellular pathways. Patients with defects in DNA damage response genes are at increased risk of cancer, including squamous cell carcinoma of the head and neck (SCCHN). In addition, haploinsufficiency for several key DNA damage response genes (ATM, H2AX, MRE11A, and CHEK1) causes chromosomal instability, one form of which, 11q13 amplification is present in ~45% of SCCHN and is an independent (poor) prognostic factor. 11q13 amplification may be tied directly to DNA damage response defects because the first step in 11q13 amplification is deletion of distal 11q, including the key DNA damage response genes listed above. We hypothesize that 11q13 amplification may cause chromosomal instability, not only due to amplification and overexpression of critical cellular genes including cyclin D1, but due to haploinsufficiency for key DNA damage response genes and the consequent defects in the cellular DNA damage response. These defects would have implications throughout the network of cellular pathways and may be responsible for the poor prognosis in SCCHN patients with 11q13 amplification. Multifaceted examination of defects in the DNA damage response is underway in our laboratories. Fluorescence in situ hybridization (FISH) has shown copy number loss of the ATM and H2AX genes compared to chromosome 11 centromere in SCCHN cell lines with 11q13 amplification. Cytogenetic analysis after treatment with DNA damaging agents is also in progress to determine whether haploinsufficiency for ATM and nearby DNA damage response genes results in increased chromosome breakage. Western blotting analysis is in progress to examine ATR and ATM signalling after DNA damage induced by ionizing or ultraviolet radiation in these cell lines. Defects in the DNA damage response in SCCHN with 11q13 amplification may have important implications for therapy, utility as diagnostic and prognostic markers and may serve as novel targets for therapeutic intervention.

7
Does Calorie Restriction Reduce Age-Related Chromosome Breakage?

Charleen M. Moore[a], Betty G. Dunn[b], C. Alex McMahan[c], Catherine L. Dunne[a], Julie A. Mattison[d], Mark A. Lane[d], George S. Roth[d], Donald K. Ingram[d]

Departments of [a] Cellular and Structural Biology, [b] Clinical Laboratory Sciences and [c] Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, [d] Laboratory of Experimental Gerontology, National Institute on Aging, NIH, Baltimore, MD, USA

Chromosome breakage has long been considered a classic example of genomic instability and a possible biomarker of aging. This has been shown in several species. Liver and kidney cells from older mice have significantly higher frequencies of chromosome aberrations than cells from younger animals. Peripheral blood lymphocytes of human subjects have an increase in chromosome aberrations with age. Human progeriod syndromes (e.g. Wer-

ner and Cockayne syndromes) have premature aging, high somatic mutation rates with numerous chromosome rearrangements, and early death from cancer or coronary artery disease.

Several studies have examined the relationship of human diet to chromosome damage and aging. Most studies, however, have examined single components of the diet (e.g. vitamin supplements or severe protein deficiencies), but none have examined the effects of overall diet.

We have examined whether the accumulation of chromosome aberrations in aging primates is reduced with long-term calorie-restriction, a nutritional intervention well known to extend lifespan and retard aging in rodents. To this end, we obtained peripheral blood samples from a longitudinal study of aging in rhesus monkeys *(Macaca mulatta)* maintained by the National Institute on Aging. For over 15 years these animals have been on a well-balanced diet, but half of the animals have been restricted to about 30% less calories than the control group. Lymphocytes were examined with conventional staining and whole chromosome paints from the first wave of mitoses obtained by initiating harvest at 42 h. The individuals analyzing the cells for breakage were blinded as to age and dietary status of the animals to prevent bias in collection of the data. With conventional staining, chromosome and chromatid gaps and breaks were recorded as well as dicentric, acentric, and ring chromosomes and exchange figures. With whole chromosome paints, cells were also examined for translocations and insertions. A description of the calorie-restricted rhesus colony and results of the chromosome breakage analysis will be presented.

This study is supported in part by a grant from the National Institute on Aging, AG-021388.

8
Genotoxic Evaluation of Methyl Tertiary Butyl Ether (MTBE), a Common Gasoline Additive, on Cultured Human Lymphocytes Exposed in vitro

Sumin Qiu[a], Bill A. Rampy[a], David K. Rassin[b], Shuliu Zhang[b], Gopalrao Velagaleti[a, b]

Departments of [a] Pathology and [b] Pediatrics, University of Texas Medical Branch, Galveston, TX, USA

Methyl tertiary butyl ether (MTBE) is an organic solvent and is commonly used as an additive to gasoline to increase oxygen content and to reduce automobile emission of air pollutants. In vivo studies using transformed tumor cell lines and in vivo animal studies indicated that MTBE is mutagenic and oncogenic. Groundwater MTBE contamination from leaking underground gasoline storage tanks was a major problem in 47 of the 50 states in the United States in 2002. The mutagenic potential of MTBE on human subjects is not known. In order to determine the mutagenic potential of MTBE on human peripheral blood lymphocytes in vitro, we have evaluated MTBE-induced genotoxicity by analysis of sister chromatid exchanges (SCE), chromosome aberrations (CA) and cell cycle progression (CCP) assays in 10 donors using two concentrations of MTBE. Increased numbers of SCEs in cells treated with MTBE at 80 ppb were detected and the numbers of SCEs were statistically significant compared to control and 40 ppb groups (8.18 vs 6.51 and 6.75, respectively, $P < 0.001$). No statistical significance was detected between 40 ppb and control groups. Similarly, the frequency of CA was significantly higher at 80 ppb compared to control and 40 ppb groups (7.2 vs 4.4 and 4.7, respectively, $P < 0.05$). No significant differences were observed among the 3 groups for CCP. However, individual donors showed significant differences in CCP at MTBE concentrations of 80 ppb ($P < 0.001$). Of interest in our study is the observation that the ethnic background of the individual donor might influence the genotoxicity of MTBE. Donors of Chinese origin have consistently shown significant differences in SCE, CA and CCP assays compared to donors of Caucasian origin. Such racial differences can be explained by the known polymorphisms for enzymes involved in the breakdown of chemicals that exist between different racial and ethnic groups. MTBE is metabolized in humans by the cytochrome P450 isozyme CYP2A6. Published literature has shown that Southeast Asians, especially Chinese and Japanese have polymorphic alleles of CYP2A6 which leads to a slow metabolizer phenotype. Based on our limited preliminary studies, we propose that MTBE is a clastogen in certain races.

9
Breakage at a Chromosomal Fragile Site May Be Associated with 11q13 Gene Amplification in Oral Cancer

Shalini C. Reshmi[a], Xin Huang[a], Robert C. Black[b], William S. Saunders[b, c, d], Susanne M. Gollin[a, c, d]

[a] Dept. of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; [b] Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, [c] The Oral Cancer Center at the University of Pittsburgh, Pittsburgh, PA, [d] University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

Double stranded breaks (DSBs) in mammalian chromosomes may be induced by various factors including ionizing radiation, chemicals, oxygen deprivation, stalled replication forks, and highly specialized cellular processes. Previous studies have suggested that stalled replication forks may represent regions containing chromosomal fragile sites, in which a chromosomal rearrangement may result from an attempt to repair the DSB. To date, eight common fragile sites have shown involvement in human cancers. Of these, two fragile sites exhibited recurrent breaks consistent with a breakage fusion bridge (BFB) cycle model for gene amplification. Previous studies from our laboratory suggest that 11q13 amplification in oral squamous cell carcinoma (OSCC) cells occurs via breakage-fusion-bridge (BFB) cycles, demonstrated by an inverted duplication containing the amplified CCND1 gene, flanked by RIN1. Following the BFB cycle model, we propose that one event leading to 11q13 gene amplification may require a distal DSB. The locus 11q14.2, which is telomeric to 11q13, has been shown to harbor the common fragile site, FRA11F. In order to localize FRA11F on the physical map, we carried out fluorescence in situ hybridization (FISH) with a series of contiguous BACs in aphidicolin-treated peripheral blood cells. To determine the relationship between this distal fragile site and the 11q13 amplicon, BACs appearing to span the FRA11F region were hybridized to OSCC cell lines previously shown to contain 11q13 gene amplification. Our preliminary data suggest that breakage at FRA11F may be an important step for 11q13 gene amplification in OSCC cells.

10
Inactivation of INI1 Distinguishes CNS Rhabdoid Tumor from Choroid Plexus Carcinoma

Jaclyn A. Biegel[a], Peter Burger[b], Ronald L. Hamilton[c], Bette Kleinschmidt-DeMasters[d], Arie Perry[e], Scott Pomeroy[f], Marc K. Rosenblum[g], Anthony T. Yachnis[h], Lucy B. Rorke[a], Alexander R. Judkins[a]

[a] Children's Hospital of Philadelphia, PA, [b] Johns Hopkins University, [c] Children's Hospital of Pittsburgh; [d] University of Colorado Health Sciences Center, [e] Washington University St Louis, MO, [f] Childrens Hospital of Boston, MA, [g] Memorial Sloan Kettering Cancer Center, [h] University of Florida at Gainesville, FL, USA

Central nervous system atypical teratoid/rhabdoid tumor (AT/RT) and choroid plexus carcinoma (CPC) are rare, highly malignant tumors that predominantly arise in young children. Overlapping clinical, histologic and immunophenotypic features may obscure the diagnosis. Rhabdoid tumors are characterized by deletions and/or mutations of the INI1 tumor suppressor gene in 22q11.2. Choroid plexus carcinomas often have hypodiploid or complex karyotypes, and may be seen in association with Li-Fraumeni syndrome. Several published but controversial studies have reported deletions of chromosome 22 or mutations of INI1 in CPC. Our goal was to determine if inactivation of INI1, as shown by negative staining with a newly described INI1 antibody, could be used to distinguish AT/RT from CPC. Twenty-four tumors with a diagnosis of CPC were subjected to histologic review and immunohistochemical analysis. Cytogenetic, FISH and INI1 mutation results were also available for a limited number of tumors. Three of the 24 tumors showed lack of staining for INI1, and in 2 of these, the histologic diagnosis was revised to AT/RT. In contrast to previous reports, we find

minimal genetic overlap between AT/RT and CPC. If the histologic diagnosis is unclear, alterations of INI1 at the genomic or protein level may be used to resolve the diagnosis and guide treatment.

11
Cytogenetic and Molecular Cytogenetic Studies on a Variant of t(21;22), ins(22;21)(q12;q22q22), in a Patient with Ewing Sarcoma

Deborah J. Hopcus-Niccum, Jiyun Lee, John J. Mulvihill, Shibo Li

Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Ewing sarcoma is one of the most malignant tumors in young people. Cytogenetic analysis to identify the common t(11;22) or less frequently t(21;22) plays a major role in making the clinical diagnosis. We report a 10-year-old female who had extraskeletal Ewing sarcoma. Conventional cytogenetic analysis of tumor cells revealed that 7 out of 20 cells showed a derivative chromosome 22, possibly due to an insertion of the long arm of chromosome 21q21→q22. Fluorescence in situ hybridization (FISH) utilizing whole chromosome paint probes of chromosomes 21 and 22 confirmed the cytogenetics diagnosis. To our knowledge this is the first case report of an insertion of a segment of chromosome 21q21→q22 into the long arm of chromosome 22q12 in Ewing sarcoma. This suggests that the insertion of chromosome 21 as another possible mechanism that can lead to EWS-ERG gene fusion.

12
Assessment of Banding Resolution for a Case

Debra Saxe, Daynna Wolff

Department of Human Genetics, Emory University School of Medicine, and Department of Pathology, Medical University of South Carolina, SC, USA

The Clinical Laboratory Information Act of 1988 (CLIA) states that each cytogenetic laboratory 'must have records that reflect the quality of banding; that the resolution is sufficient to support the reported result'. Thus, cytogenetic band resolution determination is a standard operating procedure in clinical cytogenetics. The College of American Pathologists (CAP) survey checklist includes questions related to reporting band resolution and the American College of Medical Genetics (ACMG) provides directives for appropriate band levels needed for various sample types and indications. However, there are no standards or regulations that govern band resolution assessment and reporting for an entire case. As an initial step towards developing a standardized methodology that would provide the necessary clinical information on band level resolution to allow for optimal patient care, the Southern Regional Genetics Group (SERGG) Cytogenetics subcommittee gathered pilot data from a subset of US clinical cytogenetics laboratories. Three 'cases' with two karyotypes each were provided to each of the 29 laboratories in SERGG and the laboratory directors were asked to provide the band resolution for each case and to answer several ancillary questions. The data from 18 (62%) of the laboratories surveyed revealed that all reported a band resolution estimate on at least a portion of the reports. However, no two laboratories assessed the band level resolution of an entire case in the exact same manner and estimation was considerably variable. We propose that inter- and intra-laboratory standardization of band level resolution estimation is necessary to ensure that the resolution is sufficient to support the reported cytogenetic results. We would like to discuss several methods for band level assessment of an entire case and propose a range of band levels for the analyzed or karyotyped metaphases may allow for a more accurate approach to reporting.

13
Molecular Karyotyping by Array CGH: Progress and Promise

David H. Ledbetter

Department of Human Genetics, Emory University, Atlanta, GA, USA

Genome-wide telomere screening using FISH has revealed that 3–5% of unexplained mental retardation is due to submicroscopic or cryptic telomere imbalance not detected by routine G-banding analysis. However, telomere FISH is labor-intensive and assays only a limited portion of the genome. Array based CGH (aCGH) is an alternative genome-wide approach that might be powerful and cost-effective if shown to be reliable for clinical applications. We have performed two pilot studies on different clinical populations to assess aCGH potential, including 1) unexplained mental retardation and 2) POCs (products of conception).

For patients with unexplained mental retardation and a normal G-banded karyotype, we have previously performed telomere FISH on over 500 individuals. A blinded study was designed which included 23 abnormal and 70 normal telomere FISH cases for aCGH analysis. The aCGH accurately identified all 23 abnormal cases, including 12 terminal deletions, 4 unbalanced translocations, and 4 cases of partial trisomy. Surprisingly, the aCGH study also identified 4 duplications that were not detected by FISH analysis. These included duplications on 4q and 10q telomeres, and one case with duplication of a clone from the cat-eye syndrome region on chromsome 22 (GSA 300 array).

In a second pilot study, 41 POC samples, previously analyzed by G-banding, were tested. aCGH detected all abnormalities identified by G-banding analysis and revealed additional abnormalities in 4/41 (9.8%) cases. Of these, one trisomy 21 case was also mosaic for trisomy 20, one had a duplication of the 10q telomere region, one had an interstitial deletion of chromosome 9p and the fourth had an interstitial duplication of the Prader-Willi/Angelman syndrome region on chromosome 15q.

Taken together, these two pilot studies demonstrate that aCGH has the capability to detect abnormalities currently detected by G-banding or telomere FISH plus the detection of submicroscopic or cryptic abnormalities currently missed by these standard technologies. Since aCGH is potentially more amenable to automation than genome wide FISH approaches, it may provide a more cost-effective and sensitive approach to a 'molecular karyotype'.

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14
Development and Validation of Chromosome Microarray (CMA) for Clinical Diagnosis

S.W. Cheung, C.A. Shaw, W. Yu, J.Z. Li, Z. Ou, A. Patel, P. Stankiewicz, A.C. Chinault, A.L. Beaudet

Department of Human and Molecular Genetics, Baylor College of Medicine, TX, USA

We have developed a BAC-based microarray for clinical diagnosis of genetic disorders by comparative genomic hybridization (CGH). The array contains 380 FISH-verified clones that span genome regions implicated in over 40 known human genetic disorders. Additionally, the array contains subtelomeric clones for all 41 human chromosome telomeric regions. The current array represents the second generation of work at BCM to develop human CGH arrays for diagnostic application. The initial pilot array, which was validated in 2003, consisted of 41 telomere clones and 31 clones from genomic positions corresponding to 21 different genetic disorders. When this initial array was tested in a blind study on 20 clinical cases with known aberrations previously established by the cytogenetics laboratory, all known chromosome aberrations revealed by FISH analysis were detected, as well as some additional chromosome imbalances that were not detected with the current standard practice. The expanded array represents a substantial enhancement over the pilot array. The new array has multi-clone replication for each disease, with an average of 3–4 clones representing each locus, as well as 4 or more clones for each telomere. Therefore, an additional 20

microdeletion syndromes are reflected on this array. Results from the expanded array have been consistent with previous cytogenetic findings in over 15 cases studied so far. Overall we find this to be a specific, sensitive and fast approach for detecting chromosome imbalances. The main limitation of this array is its inability to detect chromosome changes such as balanced translocations, inversions or low levels of mosaicism. However, our preliminary data suggest that a significant number of cryptic rearrangements or deletions may go undetected by typical chromosome analysis as well. Therefore, we conclude that the use of Chromosome Microarray Analysis together with standard cytogenetics can significantly improve diagnostic precision.

15
Multiple Microdeletions in a Patient with Intellectual Disability and Autism Spectrum Disorder Detected Using a 1 Mb CGH Array

C. Harvard[a], P. Malenfant[b], M. Koochek[c], J.J.A. Holden[b], M.E.S. Lewis[c], E. Rajcan-Separovic[a]

[a] Department of Pathology and [c] Medical Genetics, University of British Columbia, Vancouver, [b] Department Psychiatry & Physiology, Queen's University, Kingston, Canada

Intellectual disability is present in approximately 1–3% of the population. Many cases are believed to be caused by small chromosome imbalances such as deletions or duplications. The resolution of standard cytogenetic techniques is usually limited to 5–10 Mb, and so smaller imbalances remain undetected and the etiology of the disorder unknown. CGH microarray technology is as an exciting new technique which can increase the resolution of cytogenetic analysis over 10 times. We report results on a 13-year-old boy who presented with confirmed autism spectrum disorder, intellectual disability, dysmorphic features, and a de novo translocation with breakpoints at 5p15.1 and 7p13 and a pericentric inversion of chromosome 3 with breakpoints at 3p24 and 3q24. G banding analysis suggested that the t(5;7) was not balanced and a small segmental loss of 7p material at the breakpoint was proposed. A 1 Mb CGH microarray (Spectral genomics) was used to search for gains and losses at a higher resolution, and revealed a deletion of 1.2–4 Mb at 5p15.2. In addition, a single clone loss at the 3p24 breakpoint was noticed as well as a single clone deletion at 18q12.2. All deletions were confirmed using microsatellite analysis; FISH is in progress to determine whether they are de novo or inherited. None of these clones were deleted in 23 normal or intellectually disabled individuals also screened for chromosome abnormalities using microarrays. The specific 5p12.2 region deleted in our patient is in close proximity to the cri-du chat critical region. The patient, however, does not demonstrate any features of this syndrome.

These findings are therefore of value in further clarifying the extent of the cri du chat critical region, and will allow the genetic assessment of the newly discovered deletions of a single clone from 3p24 and 18q12.2. Interestingly, the sister of this patient had similar clinical features including confirmed autism spectrum disorder and intellectual disability; however her karyotype as well as the array CGH profile, were normal. We believe that this case demonstrates that the array-CGH technique provides a much greater insight into sub-microscopic chromosome imbalances than conventional cytogenetic techniques.

16
Comparative Genomic Hybridization Analysis of Products of Conception Reveals High Maternal Contamination Rate and Unusual Spectrum of Chromosomal Abnormalities

B.L. Lomax, C. McKenna, K. Hepburn, F. Dill, P. Eydoux

Department of Pathology, Cytogenetics Laboratory, Women's and Children's Health Centre, Vancouver, B.C., Canada

Cytogenetic analysis of products of conception (POCs) is important for establishing both the cause of pregnancy loss and the recurrence risk. Conventional cytogenetic analysis relies on culturing viable tissue. Specimens from pregnancy loss are frequently macerated and fail to grow in culture. Overgrowth of maternally-derived cells can result in a misleading 46,XX karyotype. Comparative genomic hydridization (CGH) is a DNA-based cytogenetic technique that offers advantages over conventional cytogenetic methodologies for the analysis of POCs. When combined with flow cytometry, CGH can effectively detect aneuploidy, polyploidy and unbalanced structural rearrangements and overcome the limitations of tissue culture.

We performed CGH/flow analysis of 500 POCs submitted for cytogenetic analysis when: tissue culture failed (n = 279), when no viable tissue was available for conventional analysis (i.e. specimen frozen or fixed) (n = 60), when a 46,XX karyotype was produced from cultured chorion (n = 156), or when further delineation of a chromosomal abnormality was required (n = 5).

Of 279 specimens analyzed by CGH for tissue culture failure, 209 (75%) generated results with CGH. Poor quality DNA due to tissue maceration was the principle cause of CGH failure. In 16 cases, due to small sample size, tissue was not available for flow analysis and therefore polyploidy could not be ruled out. Of 209 specimens analyzed, 103 show a balanced complement, 86 aneuploidy, 15 structural abnormalities, and 5 polyploidy.

Of 156 specimens submitted for CGH to rule out maternal contamination, 66 demonstrate aneuploidy (n = 39), polyploidy (n = 8), structural imbalance (n = 8) or a male genotype (n = 11), while 80 show a female complement. Our findings demonstrate that at least 42% of the 46,XX karyotypes generated from cultured chorion are a product of maternal cell overgrowth. Ten specimens could not be analyzed by CGH as the fetal/placental tissue generated only degraded DNA, raising further suspicion of maternal overgrowth in these cases.

Of 60 frozen or fixed specimens with no karyotype, 50 were successfully analyzed by CGH/flow.

Using CGH/flow as a supplement to conventional cytogenetic analysis, we have lowered our overall failure rate from 12 to 3% and increased the sensitivity of our analysis by eliminating most maternal contamination. In addition, we are able to study frozen and fixed specimens that would not otherwise be analyzed. Of particular interest, CGH analysis demonstrated 8 cases of monosomy 21, and a high rate of unbalanced chromosomal rearrangements (5.9%). These findings are probably related to specific chromosomal imbalances resulting in culture failure.

17
Genomic Microarray Analysis of Prostate Cancers: Problems with Cellular Heterogeneity May Finally Be Solved

Arthur R. Brothman, Joseph A. Pettus, Brett C. Cowley, Teresa Maxwell, Brett Milash, Charles Hoff, Robert A. Stephenson, L. Ralph Rohr

University of Utah School of Medicine, Salt Lake City, UT, USA

Prostate cancer remains the most common male malignancy in western countries, yet no consistent genetic changes associated with clinical outcomes have been identified. Cellular heterogeneity has been one confounding problem in previous studies of prostate tumors, since a significant portion of all adenocarcinomas contain normal prostate epithelial cells. Attempts to study abnormal cell populations have led to approaches ranging from modified growth medias and elaborate cell culturing protocols to the use of microdissection techniques. Nevertheless, few consistent cytogenetic changes have been observed in prostate tumors, and many have yielded primarily normal karyotypes. The advent of genomic microarrays (GMs) has provided an attractive alternative to conventional karyotyping, chromosomal comparative genomic hybridization and allelotyping. This technique can now detect copy number changes with a resolution covering the genome at approximately 1 Mb intervals. Our laboratory has been studying prostate cancer since the mid 1980s. We have analyzed tumors using all the above-noted methods, and also retained fresh frozen tissue on many of our specimens. DNA was prepared from 20 previously designated cancer tissues and hybridized to Spectral Genomics (Houston, TX) Spectral Chip 2600TM. Protocols were essentially as described by the manufacturer and GMs were scanned on Axon's

GenePix 4000B microarray scanner; images were analyzed with SpectralWare 2.0. Whole and partial chromosomal gains and losses were readily detected with a dramatically increased sensitivity to previous cytogenetic analyses on the same patients. These abnormalities were validated statistically by calculating the likelihood of runs of adjacent amplified or deleted clones in repeated permutations of the data; many were confirmed by FISH using BAC clones correlating with aberrations seen in the array ratio plots. The detection of minor populations in heterogeneous samples and correlation of specific abnormalities with clinical follow-up of our patients will be discussed.

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18
Developmental Genome Anatomy Project (DGAP): Cytogenetic Approaches to Gene Identification

N.T. Leach[a, e], G.A.P. Bruns[c, e], D.J. Donovan[a], R. Eisenman[c], H.L. Ferguson[a], J.F. Gusella[b, e], D.J. Harris[a, c], S.R. Herrick[a], A.W. Higgins[a, e], A.H. Ligon[a, e], H.G. Kim[b, e], K.M. Kocher[e], W. Lu[a, e], R.L. Maas[a, e], M.E. MacDonald[b, e], S. Michaud[a, e], A.M. Michelson[a, d, e], S.D. Moore[a], R.E. Peters[a], B.J. Quade[e], F. Quintero-Rivera[b, e], I. Saadi[a, e], R.E. Williamson[e], C.C. Morton[a, e]

[a] Brigham & Women's Hospital, Boston, MA, [b] Massachusetts General Hospital, Charlestown, MA, [c] Children's Hospital, Boston, MA, [d] Howard Hughes Medical Institute, [e] Harvard Medical School, Boston, MA, USA

The Developmental Genome Anatomy Project (DGAP, http://dgap.harvard.edu) represents a collaborative effort to identify genes important for human development. Breakpoints of apparently cytologically balanced chromosomal rearrangements in individuals with congenital anomalies are analyzed in an attempt to uncover potential causal relationships between rearrangements and phenotypes. A benefit to the individual is a possible explanation of the disorder that may be otherwise undefined. The high-throughput approach underway in DGAP involves: (1) patient identification and sample collection, (2) FISH-based breakpoint localization, (3) breakpoint cloning and candidate gene identification, and (4) functional analysis in model organisms. To date, more than 137 cases have been ascertained through international collaborations with clinicians, cytogeneticists and genetic counselors. Forty-two cases have been FISH-mapped in detail, with 48 breakpoints positioned on the human genome map within a single BAC clone. Nineteen breakpoints have been localized further to 0.5–53 kb, and 13 have been cloned. For 20 delineated breakpoints a known gene was disrupted. Of the 13 cloned breakpoints, 9 fall within an intronic region, none falls within an exon, one falls within a 3′UTR, and 3 fall in non-genic regions. In the three latter instances, the breakpoint on the other derivative chromosome disrupts a gene. Presence of a microdeletion at the breakpoint was found in only 2 of 48 cases. Candidate genes have been identified in 23 cases studied. Included among them is DGKD, a gene implicated in a seizure phenotype, and MTAP and FLJ21820, whose disruption is suspected in causing hearing loss. For the seven most attractive candidates, gene knock-out mouse models are being created for functional testing. Achievements made through DGAP, built on integral collaborations within the medical genetics community, will ultimately provide critical insights into human development.

19
Breakpoint Mapping of Two X;Autosome Translocations in Females with Duchenne Muscular Dystrophy (DMD)

A.C.V. Krepischi-Santos, I.E. Kerkis, A.M. Vianna-Morgante

Departamento de Biologia, Instituto de Biociencias, Universidade de Sao Paulo, SP, Brazil

Two de novo balanced translocations [t(X;4) and t(X;22)] in females with Duchenne muscular dystrophy (DMD) were studied aiming at disclosing the mechanisms of the rearrangements through mapping of the breakpoints. In both cases, the translocated X chromosome was paternal and was shown to be preferentially active. Breakpoint mapping was performed by FISH and DNA analyses in the patients' cells and in somatic cell hybrids. The X chromosome breakpoints were located within the dystrophin gene, at Xp21. The DMD gene disruption in association with the inactivation of the normal X led to the dystrophy phenotype. In the t(X;4), the X breakpoint was mapped to an 80-bp segment contained in BAC RP11-122N14, at the intron/exon 56 boundary of the gene (Xp21.2). Chromosome 4 breakpoint was mapped at 4q13.2, in a segment that spanned about 104 bp contained in BAC RP11-953P20. The X chromosome breakpoint of the t(X;22) was mapped to a 176-bp segment of the intron 47 in the DMD gene (Xp21.1), contained in BACs RP11-607K23. Chromosome 22 breakpoint was located in a 58-bp sequence, cloned in the BAC RP11-60G6. The breakpoint sequences are AT-rich and flanked by repetitive elements. The mechanisms of non-recurrent rearrangements are largely unknown. We did not find sequences sharing homology of at least 200 bp surrounding the breakpoints in each translocation, which would point to non-allelic homologous recombination. Non-homologous end joining then appears as the most likely mechanism at present. The involvement of the AT-rich sequences and flanking repetitive elements remains as a possibility.

20
DiGeorge/Velocardiofacial Syndrome (DGS/VCFS) as a Result of Adjacent-2 Segregation

Gail D. Wenger, Morgan Millard, Julie M. Gastier-Foster

Department of Laboratory Medicine, Columbus Children's Hospital, Columbus, OH, USA

The proband was first evaluated by cytogenetic and FISH analysis for DGS/VCFS at one day of age due to truncus arteriosus. Only one signal for the TUPLE1 probe was found in all metaphase cells examined. The karyotype was interpreted as normal at the 575 band level and written as 46,XY.ish del(22)(q11.2q11.2)(TUPLE1–). Cytogenetic analysis of the parents was recommended; however, they declined to be tested. Three years later, peripheral blood of the proband's older sibling was received. The family had experienced one miscarriage and one stillbirth in the interim. FISH analysis indicated the presence of two signals for the TUPLE1 probe as well as two signals for the ARSA marker, but a structural rearrangement was indicated. One chromosome 22 homolog had the normal signal pattern; the second red TUPLE 1 signal was observed just below the centromere of an acrocentric chromosome with morphology consistent with a D group chromosome, and the second green ARSA signal was observed on the distal long arm of an acrocentric chromosome with the length of a G group chromosome. Cytogenetic analysis confirmed the balanced karyotype 46,XX,t(13;22)(q12.3;q11.2) for the proband's sister. Re-examination of the proband's chromosomes and additional FISH analysis led to the amended karyotype 46,XY,+der(13)t(13;22)(q12.3;q11.2),–22.ish der(13)(D13Z1/+,D14Z1/D22Z1–,TUPLE1–,ARSA+), 14cen/22cen(D14Z1/D22Z1x3). This case represents an example of adjacent-2 segregation, the only example of adjacent-2 segregation in our laboratory during the 10-year time period 1994–2003, and highlights the importance of cytogenetic and molecular cytogenetic evaluation of family members of DGS/VCFS deletion patients.

21
Pericentric Inverted Duplication of Chromosome 22 Involving the DG/VCF and BCR Chromosome Regions

Frank S. Grass, J. Edward Spence
Carolinas Medical Center, Charlotte, NC, USA

The proximal region of chromosome 22 is highly unstable as exemplified by the several disorders associated with chromosomal rearrangements involving this area. We present a novel rearrangement with both inversion and duplication of proximal 22q. A 26-year-old G2P1 patient was referred for prenatal diagnosis because of an abnormal triple screen with an increased risk for Down syndrome. Ultrasound examination identified a twin pregnancy. Chromosome analyses on cultured amniocytes revealed a chromosome 22 with an apparent pericentric inversion with breakpoints at 22p11.2 and 22q13.1 in both twins. Chromosome studies on the parents were normal. To confirm the interpretation of pii, FISH studies were performed using the Tuple1 dual color probe to the DGCR (Vysis). The control probe at the ARSA locus at 22q13.3 was observed to be on the opposite side of the centromere from the Tuple1, confirming the inversion. Surprisingly, the DG/VCF hybridization signal appeared as a doublet which indicates that the locus was duplicated. Additional investigation was performed by application of the BCR/ABL, dual color FISH probe (Vysis). The BCR locus on chromosome 22 was also duplicated. The pregnancy continued, and the twins delivered by C-section at 30 weeks gestation. Birth weights and lengths were less than the 3rd percentile and head circumferences were less than the 25th percentile. Genetic evaluation at 4 months identified mild dysmorphia, failure to thrive and feeding difficulties but otherwise no major abnormalities. Duplications involving chromosome 22 are of three types: Cat-eye syndrome, der22q, and microduplication 22q11.2. The duplicated region involved in this case appears to span a longer segment than has been described in other rearrangements, and is associated with a pericentric inversion. The clinical features of the twins and the involvement of low-copy repeats relative to 22q rearrangements will be reviewed.

22
Pericentric inv(22): Another Low Copy Repeats (LCR) Mediated Recurrent Abnormality?

Manjunath A. Nimmakayalu[a], Beverly S. Emanuel[a], Vijay S. Tonk[b], Gopalrao V.N. Velagaleti[c]
[a] Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA, [b] Department of Pediatrics, Texas Tech University, Lubbock, TX, [c] Department of Pediatrics, University of Texas Medical Branch, Galveston, TX, USA

Chromosome 22 is the second smallest human chromosome but appears to be particularly susceptible to chromosome rearrangements. It has been suggested that genomic architecture associated with this region is responsible for such recurring abnormalities. Identification of chromosome-specific low copy repeat (LCR) elements within 22q11 led to the hypothesis that these LCRs might be responsible for the instability of this region. Recently, we studied a family where in the proband has duplication of distal chromosome 22q resulting from a pericentric inversion in his mother. The proband has several congenital anomalies including cleft lip and palate but no other organ malformations were noted. Karyotypic analysis from blood lymphocytes showed a structurally rearranged chromosome 22. Parental chromosome analysis showed the mother to be carrier of an inversion with karyotype 46,XX,inv(22)(p13q12.2). The proband's karyotype was interpreted as 46,XY,rec(22)dup(22q)inv(22)(p13q12.2).

Review of the literature showed 6 reported cases of inv(22), the majority of them originating from Guadalajara region of Mexico. While pericentric inversion of acrocentric chromosomes is extremely rare, the common occurrence of inv(22) prompted us to investigate the breakpoints in our case. FISH with probes BCR (22q11.2), D22Z1 (22cen), D22S553, D22S609, D22S942 (22q11.2) and ARSA (22q13.3) showed that the breakpoint is distal to BCR locus but was proximal to ARSA, suggesting that the breakpoint might be in LCR-F. BAC and cosmid probes 50D10 and 9c5 (proximal to LCR-F) and 80o7 and 20P18 (distal to LCR-F) showed that the proximal probes were in normal copy number while the distal probes were duplicated in the proband. This suggests that the breakpoint is near LCR-F. Analogous to the published literature that indicates that a variety of different LCRs are responsible for the deletions involved with DGS/VCFS/CAFS and duplications of CES, we propose that LCR-F which is immediately distal to BCR locus might be responsible for this pericentric inversion in our patient. Further studies are in progress to fine map the breakpoints in this case.

23
Parental Origins and Segregation Outcomes Involving the t(11;22)(q23;q11.2)

Melissa Leve, Rachael Paniagua, Jay W. Moore
Genzyme Genetics, Tampa, FL, USA

The 11;22 translocation [t(11;22)(q23;q11.2)] is the most common reciprocal translocation in man and is usually inherited from a carrier parent. Offspring may inherit two normal pairs of chromosomes 11 and 22, a balanced 11;22 translocation; unbalanced rearrangements could include inheritance of a der(11) or a der(22) resulting from adjacent-1 segregation, or a supernumerary der(22) resulting from 3:1 segregation.

We searched the Genzyme Genetics database for prenatal and neonatal samples received over the last 5 years that were found to have inherited either the balanced 11;22 translocation or one of the unbalanced forms described above to assess inheritance patterns and parental origin. Of the 77 cases identified, 50 had a balanced 11;22 translocation, 3 had inherited a der(11) and 24 had a supernumerary der(22). There were no cases identified that inherited the der(22) with a 46 chromosome count. Of the 50 cases with the balanced 11;22 translocation, 20 were determined to be maternally inherited, 13 were paternal, 6 were apparently de novo and 11 were unknown. Of the 24 cases with the supernumerary der(22), 19 were maternally inherited, none were paternal, 2 were apparently de novo in origin, and 3 were unknown. All 3 cases with the der(11) were inherited maternally.

These data reveal that of the offspring that did not inherit normal pairs of chromosomes 11 and 22 from their translocation carrier parents, 65% received a balanced translocation and 35% had unbalanced derivatives. Of the unbalanced outcomes, inheritance of the supernumerary der(22) occurred most frequently whereas presence of the der(22) with a 46 chromosome count was never observed. It is interesting to note that a total of 8 cases (10%) appeared to be de novo in origin; in addition, 25/27 unbalanced outcomes were maternal in origin and 2 were apparently de novo.

24
The Role of Chromosomes in Mammalian Evolution: Causes and Consequences

Fernando Pardo-Manuel de Villena
Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Chromosomes have dual functions. On one hand, genetic information is encrypted within the DNA molecule. On the other hand, chromosomes are delivery systems that ensure the stable and faithful transmission of this information to the products of each cell division, and, therefore, are key in maintaining the genetic integrity and identity of organisms. The chromosomes present in extant mammalian species have evolved from their common ancestor through the accumulation of a diverse collection of mutations in each phylogenetic lineage. As for any other biological character, these mutations have been subject to a variety of evolutionary forces including mutation rate, genetic drift and natural selection. The relative impact of each one of those forces and their evolutionary significance has been the subject of controversy for decades. This controversy has both practical and general implications. For example, the ancestral karyotype of the common ancestor of several mammalian lineages, such as eutherians, carnivores and primates,

has been reconstructed recently by applying basic evolutionary methods to comparative cytogenetics. Among the goals of these studies is to understand the dynamics of chromosome rearrangements and their implications in human disease. Ultimately these studies are expected to provide a comprehensive evolutionary history of every mammalian species and the forces that have shaped this process. Although the value of such studies in determining what has taken place during evolution is unquestionable, their underlying assumptions, in many cases, remain untested. Our research has focused in determining the forces that control why mutations have become fixed in some mammalian lineages. The paradigm that is emerging from these studies is that an unusual type of natural selection, known as meiotic drive, has been the major player in the evolution of the mammalian karyotype. In addition to opening new avenues for cytogenetic and molecular research, this conclusion also rekindles the controversy of the role of chromosome change in mammalian speciation. In contrast with the prevailing view, our results indicate chromosomes have played a leading role in speciation and that karyotypic evolution is not only a consequence but also a cause of mammalian evolution.

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Centromere Dynamics and Chromosome Evolution in Marsupials

Rachel J. O'Neill

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA

The eukaryotic centromere poses an interesting evolutionary paradox: it is a chromatin entity indispensable to precise chromosome segregation in all eukaryotes, yet the DNA at the heart of the centromere is remarkably variable. Its important role of spindle attachment to the kinetochore during meiosis and mitosis notwithstanding, recent studies implicate the centromere as an active player in chromosome evolution and the divergence of species. This is exemplified by centromeric involvement in translocations, fusions, inversions and centric shifts. Often species are defined karyotypically simply by the position of the centromere on certain chromosomes. Little is known about how the centromere, either as a functioning unit of chromatin or as a specific block of repetitive DNA sequences, acts in the creation of these types of chromosome rearrangements in an evolutionary context. Macropodine marsupials (kangaroos and wallabies) offer unique insights into current theories expositing centromere emergence during karyotypic diversification and speciation. Hybrids between different kangaroo species provide evidence that the centromere is unstable within this group of mammals and can be involved in a large number of chromosome aberrations.

26
Centromeric Drive in Mixed Karyotypes of *P. maniculatus* and *P. polionotus* F1, N1 and F2 Hybrids

Jennifer A. Mack, Rachel J. O'Neill

University of Connecticut, Molecular Cell Biology Department, Storrs, CT, USA

Proper chromosomal segregation during eukaryotic meiosis I and meiosis II requires the presence of a structure known as the centromere. The centromere binds to the microtubules, or spindle fibers that allow the chromosomes to segregate during division to opposite poles of the cell. Centromeric structure varies across species by size, location on the chromosome and base pair sequence, which has made the actual delineation of chromosome structure difficult. Paradoxically, the centromere's underlying function remains essential and is highly conserved. It has been suggested that this centromeric complexity could be due to genetic conflict with centromeres acting as selfish elements (Malik and Henikoff, 2002), thus ensuring that a particular centromeric structure will be paramount in a population despite harboring any deleterious effects.

The model system used to challenge this hypothesis is that of the mouse species *Peromyscus maniculatus* (BW) and *Peromyscus polionotus* (PO). Interestingly, the karyotypes of these two species share a conserved diploid number of 48, although a difference in fundamental number (number of chromosome arms) because of centromeric localization was observed between the two (62 and 83 arms, PO and BW, respectively). The ability of these two species to interbreed and produce hybrid offspring has made this system invaluable for studying chromosomal effects, in this case, the presence or absence of 'centromeric drive'. For F1 hybrids, there exists equal segregation of the parental chromosomes, however, when the offspring are backcrossed to parentals or F2 hybrids are obtained, a shift from the lower FN to that of the higher is observed. To test whether this shift to a more metacentric karyotype is due to differences in centromeric structure between the species, the centromeric protein CenpA (variant of histone 3) is currently being isolated and sequenced to determine if any change in structure is present and if one variant is preferentially segregated over the other.

27
Segmental Duplications and Genome Evolution in Marsupials

Meghan Marzelli, Gianni Ferreri, Rachel O'Neill

University of Connecticut, Storrs, CT, USA

Segmental duplications are paralogous sequences of DNA with high sequence identity. Segmental duplications can be found throughout the human genome either as interspersed sequences or in a tandem array. The presence of segmental duplications has been implicated in gene rearrangements in the human genome as well as disease susceptibility. Recently a study of human and mouse genomes has revealed approximately 300 segments of synteny, or genome similarity. Segmental duplications were found more prevalent in breaks of synteny in a comparison of the human and mouse genomes. This study suggests that segmental duplications may play an important role in genome rearrangement and evolution in eutherian mammals.

Our work focuses on characterization of a possible segmental duplication in the Macropodine *M. eugenii*. Screening of a *M. eugenii* BAC library with a previously identified Macropodine centromeric sequence, KERV-1, revealed uniform positive hybridization. Two BAC clones were further characterized (University of Arizona) and the homologous sequences were located by fluorescence in situ hybridization (FISH) on *M. eugenii* chromosomes. Each clone localized to a different chromosome within the species. Hybridization of one clone to *M. eugenii* chromosome 1 appeared to be located at the junction of two syntenic blocks represented as two separate chromosomes in another marsupial species, *A. rufescens*. Further studies using whole chromosome paints for these two *A. rufescens* chromosomes revealed a more defined location for the clone at this break in synteny. Our research will continue by subcloning this segment of DNA and identifying its flanking sequence in order to determine the extent of paralogy between these two BAC clones. This data show that segmental duplications are present in marsupials and indicates they may play a role in genome rearrangement and evolution in all mammals.

28
Chromosome Painting Applied to Testing the Basal Eutherian Karyotype

Marta Svartman[a], Gary Stone[a], John Page[b], Roscoe Stanyon[a]

[a] Comparative Molecular Cytogenetics Core and [b] Laboratory of Genomic Diversity, NCI at Frederick, MD, USA

In recent years, the proposal of a new super-ordinal grouping of extant Eutherians has been extensively explored on molecular grounds. One of the four proposed super-orders, Afrotheria, reunites morphologically very distinct species of African placentals, and has been the subject of debate. We karyotypically analyzed a species of Afrotheria, the short-eared elephant

shrew *Macroscelides proboscideus.* The karyotype of this species, including G- and C-banding patterns, as well as the Ag-NOR distribution, was described for the first time. Chromosome painting with human probes was used to further characterize this complement and to test various hypotheses of the ancestral Eutherian karyotype, which currently range from 2n = 44 to 2n = 50. Chromosome painting also allowed us to test the validity of the Afrotheria as a taxonomic assemblage. Based on our data, the most likely basal Eutherian karyotype would have 2n = 48. The finding of two human chromosome associations (1/19 and 5/21) in all Afrotheria studied to date is a cytogenetic signature of the group and gives further support to the Afrotheria assemblage.

29
A Repetitive Theme in Hybrids

Judith D. Brown, Rachel J. O'Neill

Molecular and Cell Biology, University of CT, Storrs, CT, USA

Interspecific hybrids are useful models to study genotypes and phenotypes not normally found in a species and to evaluate species-specific developmental differences. General findings among hybrid crosses are disruption of normal development and an unstable genome (e.g. hybrid sterility and inviability). Genomic instability is evident in a karyotype as chromosome deletions, amplifications or structural rearrangements. These types of changes, as well as smaller intrachromosomal aberrations and transposable element expansion may also be evident at the molecular level of a hybrid genome. Genomic instability manifested as chromosome remodeling has been observed in interspecific hybrids of insects, plants, grasshoppers, and marsupials. Chromosomal instability in eutherian *Mus* hybrids is not as well studied. There is one report of double minutes, a chromosome abnormality indicative of gene amplification, found in cell cultures derived from a 16.5-day *Mus musculus* × *Mus caroli* hybrid embryo.

Artificial insemination was used to obtain additional *Mus musculus* × *Mus caroli* hybrid embryos for genetic analysis. Subtractive hybridization techniques were then adapted for the evaluation of genetic and chromosome changes resulting from the interspecific hybridization of these species. Several suppressive subtractive hybridization techniques, including methylation-sensitive representational difference analysis, were used to target hybridization-induced genome aberrations. Characterization of clones, including cytogenetic mapping by fluorescence in situ hybridization, sequence analysis and Southern blot screening confirmed hybrid-specific difference products. Preliminary analysis of the difference products isolated using these procedures indicates a pattern of repeated sequence instability. Our data suggests repetitive DNA is prone to instability in interspecific hybrids and that the instability is often associated with an epigenetic change to the genome.

30
Investigation and Definition of the Geographical Ranges of Cytogenetically Distinct Populations of *Hyla chrysoscelis* and Their Hybrid Zones

D.S. Bonner, J.E. Wiley

Department of Pediatrics/Genetics, East Carolina University Brody School of Medicine, Greenville, NC, USA

Cope's gray treefrog, *Hyla chrysoscelis,* is a semi-arboreal amphibian found exclusively in North America. The treefrog is endemic to the eastern half of the continent from north of the Great Lakes south to the upper third of the Florida peninsula and to just west of the Mississippi River. Populations of *H. chrysoscelis* are polymorphic for the chromosomal location of the ribosomal genes. Based on these polymorphisms five separate populations have been previously described. Here we define the geographic boundaries of the separate populations and describe our investigations of the hybrid zone located between two of the groups. Silver staining was used to locate the nucleolar organizing regions (NORs), where the ribosomal genes are located. Each unique population occupies a specific range that appears to be separated by natural geographic barriers. Between two of these populations a hybrid zone was discovered and its range is now defined. Hybrids located in this zone exhibit Mendelian inheritance of NOR sites. The combinations of NOR sites seen in animals from the hybrid zone demonstrate that the hybrids are capable of reproduction. The hybrid zone appears to exist from the border of the eastern continental divide (ECD) and spreads westward into the range of a population with the species' ancestral NOR location on the short arm of chromosome 6. East of the ECD is the other parental population which has the NOR site located on the long arm of chromosome 8. The hybrid zone is relatively narrow and appears to be stable. In the southeastern portion of the range three other distinct populations are found, and contact zones for these populations are being investigated. These data and other features of the *H. chrysoscelis* karyotype suggest the possibility that *H. chrysoscelis* is actually a cryptic species complex.

31
Should There Be a Consistent System for the Reporting of Cytogenetic Abnormalities in the Medical Literature?

Kathleen A. Leppig

Genetic Services, Group Health Cooperative and Department of Pathology, University of Washington, Seattle, WA, USA

The study of human chromosomes has provided invaluable clues to the identification and cloning of disease causing genes. However, any search of the medical literature for a specific cytogenetic abnormality is complex and frequently arduous. There are inadequate systems to restrict a specific search to either constitutional or acquired chromosome abnormalities and for identifying a rearrangement as balanced or unbalanced. An aberration that results in 'one copy' of a specific cytogenetic region can be described as 'monosomy', 'deletion' or 'segmental aneusomy'. Aberrations resulting in 'three copies' of a specific cytogenetic region can be termed a 'duplication' or a 'trisomy'. The confusion is increased if there is not an accurate designation of the chromosome arm involved (p arm/short arm) and the specific bands located at the breakpoints of the rearrangement.

One proposed system to standardize the report of cytogenetic abnormalities in the medical literature could be summarized in 'cytogenetic key words'. An abnormality is minimally defined by the following: constitutional or acquired, mosaic or non-mosaic, balanced or unbalanced, chromosome and chromosome arm involved, breakpoints of the rearrangement, and whether the rearrangement causes monosomy, trisomy, tetrasomy, etc. As abnormalities are frequently multiple, this procedure would need to be expanded for each cytogenetic abnormality in a given case. The resolution of cytogenetic studies has significantly increased and is merging with molecular science. The description, location and copy number of probes used for in situ hybridization techniques to clarify cytogenetic rearrangements would be necessary as well. All medical literature with cytogenetic information should be reviewed by a cytogeneticist and comply with ISCN nomenclature.

While cytogenetic nomenclature is often confusing and complex, cytogenetics itself is often quite simple: there is either too much or too little genetic information. The challenge now is to find a format to simplify the retrieval of specific information and make results accessible to both cytogeneticists and non-cytogeneticists.

32
Correlation of Molecular and Cytogenetic Findings in 117 Pediatric Acute Leukemia Cases

W. Duey, B. Lomax, S. Maunders, K. Hepburn, J. Mathers, P.H.B. Sorensen, P. Eydoux

Cytogenetics and Molecular Pathology Laboratories, Department of Pathology, Children and Women's Health Centre of British Columbia, Vancouver, B.C., Canada

Fluorescence in situ hybridization (FISH) and molecular genetic analysis are valuable tools for diagnosis and prognosis of childhood leukemia. Genomic rearrangements, such as the TEL/AML-1 translocation, are not read-

ily detected by karyotyping and may be detected only by FISH or molecular techniques.

We present a series of 117 patients studied in our institution by both cytogenetic and molecular methods over a 3-year period. Ninety-three cases of Acute Lymphoblastic Leukemia (ALL) were studied using G-banding, FISH probes for TEL/AML-1, MLL, and BCR/ABL rearrangements and RT-PCR for the detection of TEL/AML-1 fusion. Twenty-four cases of Acute Myeloblastic Leukemia (AML) were studied using FISH probes for MLL and BCR/ABL rearrangements in addition to conventional cytogenetics.

Cytogenetic abnormalities were detected in 73/93 ALL patients and 22 of 76 patients tested by FISH with TEL/AML-1 were fusion positive. These results were comparable to the RT-PCR findings for TEL/AML-1 fusion. Six of 22 TEL/AML-1 fusion-positive patients had a normal karyotype, whereas 15/22 showed various karyotypic abnormalities. Three fusion-positive cases showed a loss of one MLL signal in addition to TEL/AML-1 fusion. Two of 93 ALL cases showed AML-1 amplification without TEL/AML-1 fusion. One case was positive for an MLL rearrangement.

Karyotype abnormalities were detected in 18/24 AML patients. Six of 21 AML cases tested by FISH showed a rearrangement of the MLL gene; all had a visible chromosomal abnormality.

All leukemias were negative for BCR/ABL fusion by FISH.

In 16/117 cases, interphase and metaphase FISH results provided additional information that aided in the overall interpretation of the genotype. Six cases showed the loss of the second TEL signal and 3 cases had additional copies of AML-1. Three TEL/AML-1 fusion-positive patients also showed loss of one MLL signal in a portion of the cells. In 3 cases, the positioning of the TEL and AML-1 signals on metaphase chromosomes aided in the interpretation of the conventional karyotype. A second TEL/AML-1 fusion signal in one case clarified the cytogenetic finding of an additional chromosome 21.

We believe that the correlation of cytogenetic, metaphase and interphase FISH results is beneficial as it provides additional information about gains or losses of chromosome segments and copy number of fusion genes. It also aids in the interpretation and clarification of conventional cytogenetic results. The accumulation of such data may in the future provide more precise diagnostic and prognostic information for children with acute leukemia.

33
An Unusual Finding in Polycythemia vera: A Case Report

S. Sastry, V. Suri

Genzyme Genetics, Yonkers, NY, USA

Polycythemia vera (PV) is a myeloproliferative disorder, associated with excessive proliferation of erythroid, granulocytic and megakaryocytic elements, all derived from a clonal expansion of a pluriotent stem cell. Cytogenetic findings of bone marrow cells in PV show clonal abnormalities, which include 5q-, 20q-, 13q- and trisomy 8 or trisomy 9. Here we present a case of an 83-year-old woman with a history of PV, with unusual cytogenetic abnormalities. She had a history of t (15;17) as was reported by another lab. Cytogenetic analysis of her leukemic blood in our lab revealed an interstitial deletion of chromosome 13 (13q14q22). However, the RB1 gene was in tact. This was supported by FISH. A second clone showed an insertion of a segment from the long arm of chromosome 17(17q11.2q23) into chromosome 14 at 14q24. FISH was performed to rule out a complex PML/RARA rearrangement. FISH analysis was performed with a dual color; dual fusion translocation probe set specific for PML gene locus 15q22 and the RARA gene locus 17q21.1 (Vysis). FISH was negative for PML/RARA rearrangement. Metaphase FISH analysis to detect a RARA gene rearrangement was performed with a dual color break apart probe containing the RARA gene locus mapped to chromosome 17q21.1 (Vysis). No evidence of a RARA rearrangement was observed. She was being treated with P32. These abnormalities could either be treatment related or could be a part of leukemic transformation.

34
The Cytogenetic Aspect of Fanconi Anemia Diagnosis

Susan B. Olson[a], Yassmine M.N. Akkari[a], Michael G. Brown[a], Blanche P. Alter[b], Neil Young[c], R. Ellen Magenis[a]

[a] Clinical Cytogenetics Laboratory, Oregon Health & Science University, Portland, OR, [b] Division of Cancer Epidemiology and Genetics, National Cancer Institute, [c] Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA

Fanconi anemia (FA) is an autosomal recessive disease characterized by congenital anomalies, bone marrow failure, and an increased risk for neoplasia. Generally, FA symptoms may include small stature, microcephaly, microphthalmia, kidney and cardiac defects, hearing loss, reduced fertility, and skin pigmentation changes. The variable phenotype in FA has made diagnosis based on clinical manifestations difficult. Diagnosis relies on a characteristic phenotype reflecting the hypersensitivity of cells to DNA cross-linking agents, including diepoxybutane (DEB), mitomycin C (MMC) and photoactivated psoralens. This is illustrated by the increased incidence of chromosome breakage and radials in metaphase cells of FA patients. In this study, we present a cohort of 951 samples referred to our clinical cytogenetics laboratory for FA testing. We have tabulated the reasons for referral and presented the test results with respect to each category of symptoms. Although FA testing has historically relied solely on DEB sensitivity, we present data on the use of a dual drug system that includes sensitivity to MMC in addition to DEB. This has allowed us to detect more FA cases, as well as providing an internal control. The data indicate the importance of performing FA testing on patients referred for aplastic anemia, and the need to use the dual drug system especially in 'grey zone' cases. The importance of testing different tissue types in cases of somatic reversion, as well as the need for periodic cytogenetic analysis of bone marrow samples from FA patients are discussed. These findings have important implications for the diagnosis and treatment of FA.

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Somatic Mosacism in Fanconi Anemia: Cytogenetic Investigation of the Dynamics of Phenotype Correction

Yassmine M.N. Akkari, Yumi Torimaru, Markus Grompe, Susan B. Olson

Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR, USA

Fanconi anemia (FA) is an autosomal recessive condition associated with congenital anomalies, progressive pancytopenia, and an increased risk for leukemia and solid tumors. Cells from FA patients are hypersensitive to DNA crosslinking agents, such as mitomycin C (MMC) and diepoxubutane (DEB), and display genomic instability. Recently, is has been recognized that the genomic instability seen in FA cells may increase the opportunity for correction of the genetic error in a proportion of the patient's blood cells by a phenomenon known as somatic reversion. In order to stimulate a somatic reversion event seen occasionally in FA, tissue samples from several FA patients were mixed in different proportions with like material from sex unmatched healthy individuals, and treated with and without MMC. Breakage analysis and fluorescent in situ hybridization (FISH) were performed to assess the resulting sensitivity and the proportion of male and female cells in the mixed samples, respectively. Our results suggest that in all blood, bone marrow, and fibroblast samples treated with MMC, the presence of only 5% healthy cells can decrease the radial percent reading to normal ranges (20% radials). In contrast to untreated mixed cultures, blood cultures treated with MMC revealed that FA cells were more prone to cell death which is consistent with the selective advantage of the reverted cell population. In addition, breakage analysis of FA cells in the normal cell milieu, as followed by FISH for female versus male cells, revealed no evidence for intercellular correction for MMC hypersensitivity. These findings have implications for the treatment of FA in general, as well as for the longterm survival of FA patients following a somatic reversion event.

36
Standards and Guidelines for Studies for Acquired Chromosome Abnormalities: Rationale and Review of Proposed ACMG Guidelines

Betsy Hirsch[a], Peter Jacky[b], Kathleen Rao[c], Daynna Wolff[d]

[a] Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN, [b] Departments of Genetics and Pathology, NW Permanente, PC, Portland, OR, [c] Department of Pediatrics, University of North Carolina, Chapel Hill, NC, [d] Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, USA

Over the past 5 years, most cytogenetics laboratories have seen a steadily increasing volume of specimens referred for analysis because of suspected or confirmed malignancies. The resulting chromosomal findings aid not only in the initial diagnosis of the disease process, but also in the selection of appropriate therapeutic regimens and in monitoring response to therapy.

Unlike a constitutional chromosome study that is typically performed a single time in a patient's lifetime, a study aimed at identifying an acquired chromosome abnormality may be undertaken several times during the lifetime of a patient diagnosed with a malignancy. The analytic variables (e.g. number of cells analyzed, number of cells screened) may be different dependent upon the clinical scenario (e.g. a pre-transplant versus post-transplant setting, an initial diagnostic work-up versus a routine clinical follow-up). Accordingly, the turn-around times that are important for patient care may differ.

The Cytogenetics subgroup of the Laboratory Quality Assurance Committee of the American College of Medical Genetics has significantly expanded and revised the Standards and Guidelines for Acquired Chromosome Abnormalities to address the unique characteristics of cancer chromosome studies, and to help laboratories maximize the patient-care value of these studies.

This presentation will include a summary of the major points of the newly proposed Guidelines including sample procurement, tissue culture, case analysis, karyotype documentation, and turn-around time recommendations for hematologic and solid tumors. The rationale for each of the major revisions to the existing Guidelines will be discussed along with selected case scenarios demonstrating clinical ramifications of the cytogenetic findings. Finally, the process for amending Standards and Guidelines for the ACMG will be presented, with emphasis on opportunities for input from the cytogenetics community at large.

37
The Genetics Laboratory Testing Algorithm in CML: Chromosomes, FISH and/or RT-PCR?

Robert Gasparini

Trabuco Canyon, CA, USA

Chronic myelogenous leukemia (CML) and a subset of acute lymphoblastic leukemia (ALL) are characterized by a classic translocation: t(9;22)(q34.1;q11.2). With the advent and subsequent FDA approval of STI-571 or Gleevec, CML is now one of a handful of diseases in which the question asked of the laboratory by the referring physician has evolved from a simple diagnostic question to a more complicated predictive question. The laboratory's ability to predict a patient's response to a specific therapeutic has started the testing community on a march towards genomic testing/medicine and has the potential to become the 'gold standard' in laboratory testing.

Complicating the CML picture are those patients that have been reported with sub-microscopic deletions on both the der(9) and the der(22) chromosome. These patients have been getting more and more scrutiny in the literature since Sinclair's 2000 Blood article. These deletions appear to be associated with resistance to treatment and poor clinical outcome putting these patients into a higher risk category. This begs the (simple) question: what is the better test to perform in the genetics laboratory for CML patients?

Using CML as a model, the evolution of laboratory testing from diagnosis to prediction and the march towards genomic medicine and ultimately new treatments for CML patients will be explored. Analytical sensitivities and specificities across methodology (chroms, FISH and RT-PCR) will be compared as well as an overview of the various commercially available CML FISH tests currently on the market including S-FISH (Vysis); ES-FISH (Vysis); D-FISH (Vysis, Ventana, Cancer Genetics) and F-FISH (Cancer Genetics). Ultimately, the goal for testing all hematopoietic disorders including CML will be the development of highly sensitive, highly specific, high throughput and cost-effective testing algorithms.

38
X/Y FISH Analysis: Manual Counting versus the Applied Imaging Spot® Counting System

Denise I. Quigley, Eric R. Hall, Barbara K. Goodman

Duke University Health System, Department of Pathology, Durham, NC, USA

Opposite sex bone marrow transplant engraftment is often monitored by assaying for sex chromosome complement in peripheral blood or bone marrow cells. FISH probes specific for chromosomes X and Y are applied and interphase nuclei are scored to determine the relative donor and recipient cell contribution in a given patient sample. Applied Imaging has developed the SPOT® counting system for automated scoring of FISH samples such as the X/Y engraftment assay. Cytovision® software applies user defined parameters to score interphase nuclei for sex chromosome complement. The user can then view the data collected by the software for accuracy and when appropriate reclassify scored cells. We conducted a side-by-side comparison of results obtained via routine manual scoring with those obtained using the SPOT® system and obtained highly consistent results. The SPOT® system is relatively user friendly and results are easy to review. We found that perhaps the most critical component of using this system is consistent sample quality and slide preparation. Advantages to using the SPOT® system include reduced hands-on technologist time compared with manual scoring and the ability to score a greater number of interphase nuclei per sample (500 compared with 200).

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FISHing for Cancer Cells in Urine

D.J. Wolff, J. Laudadio, C. Felicissimo, B. Rizdon, R. Hoda, T. Keane

Medical University of South Carolina, Charleston, SC, USA

The Vysis UroVysion Bladder Cancer Recurrence kit (UroV) consists of DNA probes that are used to detect chromosomal abnormalities frequently associated with urothelial cancer (UC). Over an 8-month period (Jan 02–Aug 02), we processed 316 samples (86% urine, 11% bladder wash, 2% other) by UroV with an overall positive rate of 22%. Of the 69 cases with positive results, all but three exhibited signals consistent with aneuploidy for chromosomes 3, 7, and 17. While UroV is FDA-approved only for bladder cancer recurrence detection, 58% of our cases had FISH testing as a screen for primary UC. When possible, UroV results were correlated with cytology and histology/surgical diagnosis. FISH and cytology results were concordant in 83% of cases (UroV neg/cytol neg = 181; UroV+/cytol + = 12) and discrepant in 17% (UroV +/cytol neg = 41; UroV neg/cytol + = 4); 6 cases had insufficient cells for FISH analysis. A total of 32 patients had surgical pathologic data available. Of the 25 patients with documented UC, 23 had at least one positive FISH test (sensitivity of 92%) and 9 had a least on positive cytology result (sensitivity of 36%). However, it is important to note that 14 (55%) of these patients also had at least one negative UroV result during this time interval. Of the 7 patients with consistently negative biopsies, 4 had positive FISH (specificity of 42%). These patients may develop detectable UC in the future, as FISH has been shown to be an early indicator for disease. However, our clinical experience with UroV has revealed that false positives may occur due to misclassification of polyploid 'umbrella' cells with a 4R4Gr4A4Go

signal pattern or due to aneuploidy unrelated to bladder cancer (e.g., epithelial cells entering bladder from fistula in patient with Crohn's disease or inadvertent seminal vesicle cells). We conclude that UroV provides for sensitive detection of aneuploidy that is often associated with urothelial carcinoma; however, clinical correlation of results is imperative given the rate of false-negative and false-positive results.

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An Issue with Internal FISH Probe Validation?

Rafael D. Holguin, Julie Sanford Hanna

Sacred Heart Medical Center, Spokane, WA, USA

Internal validation of new commercial FISH (fluorescent in situ hybridization) probes is a vital, routine process for cytogenetic laboratories. 'What specimens to use?' 'Are there positive control specimens available?' 'How does the probe perform?' are some of the questions that enter into probe validation. Our lab was receiving test requests to confirm/rule out Ewing Sarcoma (ES), so when the EWSR1 probe became commercially available, we purchased the probe. The EWSR1 probe is a dual color, dual fusion break-apart probe which hybridizes to 22q12. In a specimen with either a t(11;22) or variant, one of the two fusion signals breaks apart, yielding a 1R1G1F (one red, one green, one fusion) signal pattern. The t(11;22) or variant is found most frequently in patients with ES.

Our lab uses strict guidelines in new probe validation. Fortunately, we possessed a cytogenetically abnormal solid tumor ES specimen, with the result reported as 46,XY,t(11;22)(q24;q12)[6], to run as a positive control for this new probe. We used known cytogenetically normal bone marrow specimens to run as our negative controls. However, our positive control did not perform the way we expected. Interphase cells from the tumor were displaying a normal signal pattern according to probe specifications. We analyzed metaphases from the tumor by FISH and discovered that the whole EWSR1 probe was translocated to the derivative 11 chromosome. Several questions surfaced: 'Was the probe performing properly?', 'Did the patient carry a constitutional translocation?', 'Did the patient have ES as cytogenetic and pathology results indicated?' The importance of specimen election for use as controls is one issue that has arisen. Another issue, and one that is just as important, is what to do with findings when they conflict with previously reported results. Should the patient/clinician be informed after the final report has been given? What are the ramifications for the laboratory performing the testing/validation?

41
Detection of Translocations Specific for Burkitt's Lymphoma with MYC and MYC/IGH Probes

Hana Aviv, Ivana Maxwell, Patricia De Angelo, Elizabeth Sullivan, Angela Gonzalez

Center for Human and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ, USA

The 8;14 translocation is a highly specific rearrangement in Burkitt's lymphoma (BL) and plays a key role in its pathogenesis. This translocation and its variants, the 2;8 and 8;22 translocations, share a break at 8q24 where the human c-myc oncogene is located. The c-myc oncogene relocates to the derivative chromosome 14 in the 8;14 translocation but remains on chromosome 8 in the variant translocations. The location of the breakpoints within c-myc can therefore vary greatly and range over several hundred megabases.

We explored the ability of commercially available probes to detect the classical and variant translocations in patients with BL. We analyzed a variety of samples with the IgH/MYC triple color, dual fusion probe and the MYC dual color split-apart probe (Vysis, Downer's Grove, IL). We share our experience in the analysis of 4 of these patients. Two patients had the 8;14 translocation, one the 8;22 and one the 2;8 translocation. All translocations could be detected with the MYC split-apart probe but only the classical translocation could be detected with the IgH/MYC probe. However, use of the MYC split-apart probe could not distinguish among the translocations. Additional hybridizations, chromosome analysis or a combination of both were necessary to clarify the results. It is not always possible to perform multiple additional studies, especially if the size of specimen is limited or if analyzable metaphases are not obtained.

We conclude that using the IgH/MYC probe is problematic for detection of BL translocations. Although all 3 translocations can be detected using the MYC split-apart probe, the translocations cannot be distinguished from one another and additional tests must be performed. We welcome suggestions for designing the best strategy for FISH analysis of samples from patients with suspected BL.

42
Importance of Review for Cooperative Group Cytogenetic Studies, a Report from the Children's Oncology Group (COG)

Nyla A. Heerema, Warren Sanger, Betsy Hirsch, M. Ellen Magenis, Loris McGavran, Shivanand Patil, Kathleen Rao, Diane Roulston,

The Ohio State University, University of Nebraska, University of Minnesota, Oregon Health Sciences, University of Colorado, University of Iowa, University of North Carolina and University of Michigan

The adult cooperative cancer groups, Cancer and Leukemia Group B (CALGB), Eastern Cooperative Oncology Group (ECOG) and Southwestern Oncology Group (SWOG), as well as the children's cooperative groups, formerly the Children's Cancer Group (CCG) and the Pediatric Oncology Group (POG), now the COG, collect institutional cytogenetic results for use in correlative studies of leukemias and lymphomas. In all groups, these cytogenetic results, including karyotypes and data about processing and analysis, are centrally reviewed by at least 2 experienced cancer cytogeneticists to ensure the accuracy of data and to maintain a highquality cytogenetics database. The CCG has collected data to identify the types and frequencies of changes in interpretation made by reviewers. Missed or incorrect identification of abnormalities was classified as a major change. Band refinement or nomenclature correction was classified as a minor change. Other classifications of changes were: normal → abnormal; abnormal → normal; normal → reject; abnormal → reject, and reject → abnormal. When the institutional laboratory interpretation was unknown, that was noted. Below are the results detailing the importance of review in recent CCG studies (similar data from POG studies are not available).

	AML 2961	ALL 1961	ALL1991	ALL1952
Cases reviewed	807	1,844	1,745	1,864
Revisions	253	883	816	964
With revisions	31%	48%	47%	52%

Almost half of the results for ALL cases and nearly one-third of AML cases were modified by the reviewers. Types of changes for ALL cases in descending frequency were: normal → reject; minor changes; abnormal → reject; major changes; normal → abnormal; abnormal → normal, and reject → abnormal. Among recent ALL studies, there appeared to be a trend in which reviewers rejected fewer cases with cytogenetic findings of both normal and abnormal as described by the laboratory. Changes for AML in decreasing frequency were: abnormal → reject; minor changes; normal → reject; major changes; normal → abnormal, and abnormal → normal. Compared with ALL fewer AML cases reported as normal were rejected by the reviewers.

These results indicate that review is required to maintain a high-quality cytogenetics database in a cooperative group. A degree of accuracy data is necessary for all scientific and clinical correlations.

List of Participants

Yassmine Akkari
Oregon Health & Science University
3181 SW Sam Jackson Park Rd, MP 350
Portland, OR 97239
(503) 494-8336
akkariy@ohsu.edu

Kam Au
Diagnostic Cytogenetics
1525 13th Ave
Seattle, WA 98122
(206) 328-2026
kam@cumminsstudio.com

Hana Aviv
2123 UMDNJ-New Jersey Medical School
Cytogenetics, MSB B621
185 South Orange Avenue
Newark, NJ 07103
(973) 972-4480
avivha@umdnj.edu

Charles (Dana) Bangs
Cytogenetics Laboratory, Rm H1517
300 Pasteur Dr.
Stanford Hospital and Clinics
Stanford, CA 94305
(650) 725-7476
dana.bangs@medcenter.stanford.edu

Liming Bau
Cincinnati Children's Hopital Med. Center
3333 Burnet Ave/Human Genetics
Cincinnati, OH 45229
(513) 636-4321
liming.bau@cchmc.org

Sue Ann Berend
Genzyme Genetics – SF
2000 Vivigen Way
Santa Fe, NM 87505
(505) 438-2280
sue.berend@genzyme.com

Joy Berkley
6 Ortiz Lane
Santa Fe, NM 87508
(505) 466-9856

Robert Best
University of South Carolina School of Medicine
Two Richland Medical Park #301
Columbia, SC 29203-6808
(803) 779-4928
best@medpark.sc.edu

Jaclyn Biegel
Children's Hospital of Philadelphia
Room 1002 Abramson
3615 Civic Center Blvd
Philadelphia, PA 19104
(215) 590-3856
biegel@mail.upenn.edu

Mary Black
Oregon Health & Science University
Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790

Donna Blackwell
8921 Half Moon Ct. #202
Raleigh, NC 27613
(949) 713-3927
dblwellxx@aol.com

Diane Bonner
East Carolina University
Dept. Pediatrics/Genetics 3N66
600 Moye Blvd.
Greenville, NC 27858
(252) 744-2525
bonnerd@mail.ecu.edu

Arthur Brothman
University of Utah Health Sciences Center
3209 East Davinci Drive
Salt Lake City, UT 84121
(801) 581-5524
art.brothman@genetics.utah.edu

Judy Brown
University of Connecticut
Dept. of Diagnostic Genetic Sciences
358 Mansfield Rd. U-2101
Storrs, CT 06269-2101
(860) 486-3043
judy.brown@uconn.edu

Mike Brown
Oregon Health & Science University
Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790
brownmic@ohsu.edu

Helene Bruyere
Cytogenetics Lab, Rm 1815 JPPS1
899 West 12th Ave
Vancouver, BC V6R 3M2
(604) 875-4129
hbruyere@vanhosp.bc.ca

Eileen Bryant, Ph.D
Fred Hutchinson Cancer Research Center
1100 S. Fairview Ave
P.O. Box 19024
Seattle, WA 98109-1024
(206) 667-4257
ebryant@fhere.org

Athena M. Cherry
Stanford University Medical Center
300 Pasteur Dr., Rm #H1517
Stanford, CA 94305
(650) 723-4923
athena.cherry@medcenter.stanford.edu

Sau W. Cheung
Baylor College of Medicine
One Baylor Plaza NAB 2015
Houston, TX 77021-2024
(713) 798-6555
scheung@bcm.tmc.edu

Tina Christiansen
Olympus America, Inc.
2 Corporate Center Drive
Melville, NY 11747
(631) 844-5055
tina.christiansen@olympus.com

Uwe Claussen
Institute of Human Genetics
and Anthropology
Friedrich Schiller University
Kollegiengasse 10
DE–07740 Jena (Germany)
+49 3641-935501
ucla@mti-n.mti.uni-jena.de

Craig Davis
Oregon Health & Science University
Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790
daviscr@ohsu.edu

Sheila Dobin
Scott and White Hospital – Cytogenetics
2401 S. 31st
Temple, TX 76508
(254) 724-3704
sdobin@swmail.sw.org

KARGER
Fax + 41 61 306 12 34
E-mail karger@karger.ch
www.karger.com
© 2004 S. Karger AG, Basel
Accessible online at:
www.karger.com/cgr

Timothy Donlon
The Queen's Medical Center/Genetics
1010 S. King St, Suite 201
Honolulu, HI 96814
(808) 591-1183
donlon@hawaii.edu

Wendy Duey
Children & Women's Health Centre of BC
Cytogenetics Lab-R2085, 4480 Oak St
Vancouver, BC V7H 3V4
(604) 875-2345
eduey@cw.bc.ca

Constance Durum
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790
duramc@ohsu.edu

Thomas Frazier
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790

Robert Gasparini
21932 via del Lago
Trabudo Canyon, CA 92679
(949) 709-0520
bobgasparini@cox.net

Susan Gollin, Ph.D
University of Pittsburgh
Prof., Dept. Human Genetics
130 Desoto St.
Pittsburgh, PA 15261
(412) 624-5390
sgollin@hgen.pitt.edu

Barbara K. Goodman
Johns Hopkins University Dept. of Ob/Gyn
600 N. Wolfe St, Park Bldg B240
Baltimore, MD 21287
(919) 684-6426
barbara.goodman@duke.edu

Frank S. Grass
Parke Cytogenetics Laboratory
Carolinas Medical Center
1000 Blythe Blvd
Charlotte, NC 28232
(704) 355-3159
fgrass@carolinas.org

Amy Hanlon-Newell
OHSU
3181 SW Sam Jackson Park RD, MP 350
Portland, OR 97239
(503) 494-8336
hanlonne@ohsu.edu

Julie S. Hanna
Sacred Heart Medical Center
101 W. 8th Ave
Spokane, WA 99220
(509) 474-4414
hannaj@shmc.org

Nyla Heerema
Ohio State University
Clinical Pathology/Cytogenetics
1645 Neil Ave, 167 Hamilton
Columbus, OH 43210
(614) 292-7815
heerema-1@medctr.osu.edu

Jill Hendrickson
Molecular Probes, Inc
29851 Willow Creek Rd
Eugene, OR 97402-0469
(541) 335-0356
jill.hendrickson@probes.com

Michele Hibbard
US Laboratory
2601 Campus Drive
Irvine, CA 92612
(949) 950-0195
mhibbard@uslabs.net

Charles Hildreth
Applied Spectral Imaging, Inc
1497 Poinsettia Ave, Suite 158
Vista, CA 92081
(800) 611-3466 ext 38
charles@spectral-imaging.com

Eleanor Himoe
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790
himoee@ohsu.edu

Betsy Hirsch
University of Minnesota Med School
Dept. Lab Medicine & Pathology
420 Delaware St SE, MMC 609
Minneapolis, MN 55455
(612) 273-4952
hirsc003@tc.umn.edu

Cindy Hohenleitner
Genzyme Genetics – SF
2000 Vivigen Way
Santa Fe, NM 87505
(505) 438-2123
sch1@cybermesa.com

Rafael Holguin
Sacred Heart Medical Center
West 101 8th Ave
Spokane, WA 99203
(509) 474-4415

Deborah Hopcus-Niccum
Univ of Oklahoma Health Sciences Ctr.
941 Stanton L Young BSEB Rm. 224
Oklahoma City, OK 73104
(405) 271-3589
deborah-hopcus@ouhsc.edu

Patricia N. Howard-Peebles
1457 Cattle Baron Ct.
Fairview, TX 75068
(972) 549-0780
phpeebles@yahoo.com

Bing Huang, MD, Ph.D
Genzyme Genetics
1054 Town & Country Rd.
Orange County, CA 92686
(714) 796-2532
bihuang@genzyme.com

Colleen Jackson-Cook
Virginia Commonwealth Univ.-Pathology
PO Box 980662
Richmond, VA 23298
(804) 828-9632
jacksonc@hsc.vcu.edu

Peter Jacky, Ph.D, FACMG
Kaiser Permanente
10220 SE Sunnyside Rd
Clackamas, OR 97015
(503) 571-5633
peter.jacky@kp.org

Mehdi Jamehdor, MD
Kaiser Pemanente Genetic Testing
Genetic Testing Laboratory
4580 Electronics Place
Los Angeles, CA 90039
(818) 502-5959
mehdi.r.jamehdor@kp.org

Kathleen A. Kaiser-Rogers
University of North Carolina, Chapel Hill
5011 Thurston – Bowles Bldg. CB #7220
Chapel Hill, NC 27599
(919) 966-1595
kkr@med.unc.edu

Dogmar Kalousek
Retired Emeritus Prof. UBC
3168 West 999 Ave
Vancouver, BC V6N 3K6
(609) 263-0561
dago.sek@shaw.ca

Hutton Kearney
University of North Carolina
5011 Thurston Bowles Bldg, CB 7220
Chapel Hill, NC 27599
(919) 966-1595
hutton@med.unc.edu

Elisabeth Keitges, Ph.D
DynaCare/Lab of Pathology of Seattle
819 Boylston
Seattle, WA 98104
(206) 386-6166
ekeitges@dynagene.com

JoAnn C. Kelly
Quest Diagnostics – Cytogenetics
7600 Tyrone Ave
Van Nuys, CA 91405
(818) 376-6068
joey.c.kelly@questdiagnostics.com

Uli Klingbeil
Metasystems
32 Hammond Rd
Belmont, MA 02478

Harold P. Klinger
Albert Einstein College of Medicine
Department of Molecular Genetics
1300 Morris Park Ave
Bronx, NY 10461
(718) 430-2451
hklinger@aecom.yu.edu

Anita Kulharya
Medical College of Georgia
Augusta, GA 30912
(706) 721-3949
akulhany@mail.mcg.edu

Gloria Ladiges
Kaiser Permanente, Cytogenetics
10220 SE Sunnyside Rd
Clackamas, OR 97015

Olivia Lamb
Kaiser Permanente, Cytogenetics
10220 SE Sunnyside Rd
Clackamas, OR 97015
(503) 571-5630

Helen Lawce
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790
lawceh@ohsu.edu

Natalia Leach
77 Avenue Louis Pasteur
NRB, Suite 160
Boston, MA 02115
(617) 525-4540
nleach@rics.bwh.harvard.edu

David Ledbetter
Emory Univ. School of Med-Human Gen.
615 Michael St, Suite 31
Atlanta, GA 30322
(404) 727-3875
dledbetter@genetics.emory.edu

Kathy Leppig, MD
Genetic Services
201 16th Ave E, CMB A504 C
Seattle, WA 98112
(206) 326-2272
leppig.k@ghc.org

Melissa Leve
Genzyme Genetics
10421 University Center Drive, Suite 100
Tampa, FL 33612
(813) 615-4300

Jessica Lira
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790
liraj@ohsu.edu

Brenda Lomax
Children & Women's Health Centre BC
Cytogenetics Lab-RI 2085, 4480 Oak St.
Vancouver, BC V7H 3V4
(604) 875-2345
blomax@cw.bc.ca

Frederick Luthardt
DynaCare/Laboratory Pathology of Seattle
819 Boylston
Seattle, WA 98104
(206) 386-6166
fluthardt@dynagene.com

Jennifer Mack
University of Connecticut
354 Mansfield Rd U Box 2131
Storrs, CT 06268
(486) 860-3043
jennmack70@aol.com

R. Ellen Magenis
Oregon Health & Science University
Dept. Molecular/Medical Genetics, MP350
3181 SW Sam Jackson Park Rd
Portland, OR 97239
(503) 494-2794
magenise@ohsu.edu

Dave Marr
Applied Imaging Corporation
2380 Walsh Ave, Bldg B
Santa Clara, CA 95051
(408) 450-4352
bjones@aicorp.com

Meghan Marzelli
Dept. of Molec/Cell Biology, UCONN
354 Mansfield Rd, RM 323 U-2131
Storrs, CT 06269
(860) 486-3043
megmarz@yahoo.com

Susan Matthew
Weill-Cornell Medical Center
525 E. 68th St.
New York, NY 10021
(212) 746-4146
sum2001@med.cornell.edu

Ivana Maxwell
UMDNJ-New Jersey Medical School
Cytogenetics, MSB B621
185 South Orange Avenue
Newark, NJ 07103
(973) 972-4480
maxweliv@umdnj.edu

Suzanne Merrick
Applied Imaging
2380 Walsh Ave, Bldg. B
Santa Clara, CA 95051

Karen Montgomery
Genzyme Genetics
2000 Vivigen Way
Sante Fe, NM 87505
(800) 848-4436
karen.mmtgomery@genzyme.com

Charleen M. Moore
University of Texas Health Science Center
Dept. of Cellular and Structural Biology
7703 Floyd Curl Drive
San Antonio, TX 78229-3900
(210) 567-3875
moorec@uthscsa.edu

Lisa Moroz
Genzyme Genetics – SF
2000 Vivigen Way
Santa Fe, NM 87505
(505) 466-9856

Peter Mousseau
Rainbow Scientific, Inc.
83 Maple Ave
Windsor, CT 06095
(860) 298-8382
info@rainbowscientific.com

Thomas Norwood
University of Washington, Dept. of Pathology
Box 357470, 1
Seattle, WA 98195
(206) 685-1569
hyno@u.washington.edu

Rachel O'Neill
University of Connecticut, Dept. Molec/Cell Biology
U-131, Beach Hall
Storrs, CT 06269
(860) 486-6031
roneill@uconnvm.uconn.edu

Kent Opheim, Ph.D
Children's Hospital Medical Center
4800 Sand Point Way, NE, CH-37
Seattle, WA 98105
(206) 987-2571
opheim@u.washington.edu

Susan Olson
OHSU
3191 SW Sam Jackson Park Rd, MP 350
Portland, OR 97239
(503) 494-5964
olson@ohsu.edu

Cynthia Osborn
Kaiser Permanente, Cytogenetics
10220 SE Sunnyside Rd
Clackamas, OR 97015
(503) 571-5630

Rachel Paniagua
Genzyme Genetics
10421 University Center Drive, Suite 100
Tampa, FL 33612
(813) 615-4333
rpaniagua@tampabay.rr.com

Fernando Pardo-Manuel de Villena
Dept. of Genetics, CB# 7264
Neurosciences Research Bldg. Rm 4109B
103 Mason Farm Rd
University of North Carolina
Chapel Hill, NC 27599-7264

Linda Pasztor
Sonora Quest Laboratories
1255 W. Washington
Tempe, AZ 85281
(602) 685-5349
linda.pasztor@bannerhealth.com

Anakita Patel
Baylor College of Medicine
One Baylor Plaza, NAB 2015
Houston, TX 77030
(713) 798-8534
ankitap@bcm.tmc.edu

Shivanand Patil
University of Iowa Hospitals
W101 GH, 200 Hawkins Dr.
Iowa City, IA 52242
(319) 356-3877
shivanand-patil@uiowa.edu

Claire Pike
US Labs
2601 Campus Drive
Irvine, CA 92612
(949) 450-0145
cpike@uslabs.com

Jean Priest
Emory University, Med-Ped. Genetics
843 Barton Woods Rd, NE
Atlanta, GA 30307-1305
(404) 378-3148
jpriest517@aol.com

Norma Pucci
Chroma Technology Corporation
10 Imtec Lane, PO Box 489
Rockingham, VT 05101
(800) 824-7662
npucci@chroma.com

Denise Quigley
Duke University Health System
Dept. Pathology, Box 3631
Durham, NC 27710
(919) 613-8426
denise.quigley@duke.edu

Evica Rajcan-Separovic
Care Center of BC, Cytogenetics/Pathology
4480 Oak Street
Vancouver, BC, Canada
(604) 875-3929
esgparovic@cw.bc.ca

Nagesh Rao
UCLA School of Medicine
1000 Veteran Ave, #2-113A
Los Angeles, CA 90095
(310) 794-7851
nrao@ucla.edu

Shalini Reshmi
University of Pittsburgh, GSPH, A300 Crabtree Hall
130 DeSoto St
Pittsburgh, PA 15261
(412) 624-5358
sc549@pitt.edu

Birgitte Roland
Alberta Children's Hospital Unv of Calgary
1820 Richmond Rd. SW
Calgary, Alberta T2T 5C7, Canada
(403) 943-2216
birgitte.roland@cls.ab.ca

Reza Saleki
Signature Genomic Laboratories
44 West 6th Ave, Suite 202
Spokane, WA 99204
(509) 474-6840
saleki@signaturegenomics.com

Sudha Sastry
Genzyme Genetics – Yonkers
100 Corporate Drive
Yonkers, NY 10701
(914) 969-3399
sudasastry@aol.com

Debra F. Saxe
Emory Genetics Laboratory
2711 Irvin Way, Suite 111
Decatur, GA 30030-1724
(404) 297-1504
dsaxe@genetics.emory.edu

Rhona Schreck
Cedars-Sinai Medical Center
8700 Beverly Blvd, SSB 159
Los Angeles, CA 90048-0750
(310) 423-2878
rhona.schreck@cshs.org

Margaret Seper
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2790
seperm@ohsu.edu

Patricia Shadoan
ICDL
2220 Penitentiary Rd, Suite 16
Boise, ID 83713
(208) 343-4959
shadoanp@slrn.org

Lisa Shaffer, Ph.D
WSU Health Research/Education Center
PO Box 1495
Spokane, WA 99210-1495
(509) 368-6710
lshaffer@wsu.edu

Joann Sherman
Kaiser Permanente, Cytogenetics
10220 SE Sunnyside Rd
Clackamas, OR 97015
(503) 571-5630

Ming Shi
Genzyme Genetics
2000 Vivigen Way
Santa Fe, NM 87505
(505) 438-2158
mingshi99@hotmail.com

Cheree Smith
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 227-2829
smithcher@ohsu.edu

Guoxian Sun
Impath Inc.
521 West 57 St, 5th Floor
New York, NY 10019
(212) 485-0836
guoxian.sun@impath.com

Urvashi Surti, Ph.D
University of Pittsburgh
Cytogenetics Laboratory
300 Halket St.
Pittsburgh, PA 15213-3180
(412) 641-4267
usurti@mail.magee.edu

Marta Svartman
National Cancer Institute at Frederick
Bldg 560, RM 11-75 Fort Detrick
Fredrick, MD 21702
(301) 846-5234
svartmanm@ncircrf.gov

Karen Swisshelm
University of Washington
Pathology, Box 357470
1959 NE Pacific St
Seattle, WA 98195-7470
(206) 616-3182
kswiss@u.washington.edu

Sylvie Szpiro-Tapia
Pasteur Cerba Laboratory
95066 Cergy Pontoise, CX 9, France
+33 1344-02087
stapia@pasteur-cerba.com

Eappen Thomas
Great Lakes Genetics
2323 N. Mayfair Rd, Suite 410
Milwaukee, WI 53226
(414) 475-5904

Horace Thuline
16353 129th Ave SE
Renton, WA 98058
(425) 228-2936

James Tepperberg
Laboratory Corporation of America
1904 Alexander Drive
Research Triangle Park, NC 27709
(919) 361-7783
tepperj@labcorp.com

Janice Tessin-Thuline
16353 129th Ave SE
Renton, WA 98058
(425) 228-2936

Niels Tommerup
Kobenhavns Univ/Panum Institute
AFD Med Gen/Inst. Med Biochem
Blegdamsvej 3
2200 Kobenhavn N, Denmark
+45 3532-7826
tommerup@imbg.ku.dk

Vijay S. Tonk
Texas Tech University
Health Sciences Center
3601 4th Street
Lubbock, TX 79430
(806) 743-4243
vijay.tonk@ttuhs.edu

Jeffrey Trinklein
Kaiser Permanente, Cytogenetics
10220 SE Sunnyside Rd
Clackamas, OR 97015
(503) 571-5630

Caroyln Trunca
The Genetics Center
48 Route 25A, Suite 205
Smithtown, NY 11787
(631) 862-3620
ctrunca@thegenetics.center.com

Karen Tsuchiya
Children's Hospital and Regional Med. Ctr.
4800 Sand Point Way NE
Dept. of Labs/ 6P-1
Seattle, WA 98105
(206) 987-4937
karen.tsuchiya@seattlechildrens.org

Mary Tunby
Shodair Hospital
2755 Colonial Drive, PO Box 5539
Helena, MT 59604
(406) 587-4613
marytunby@aol.com

Gopalrao V.N. Velagaleti
University of Texas Medical Branch
Dept. of Pediatrics, 301 University Blvd
Galveston, TX 77573
(409) 772-3466
govelaga@utmb.edu

Angela Vianna-Morgante, Ph.D
University Sao Paulo, Dept. Biology
CP 11461
05422-970 Sao Paulo, Brazil
+55-11-3091-7591
avmorgan@ib.usp.br

Gail D. Wenger
Children's Hospital Cytogenetics Laboratory
700 Children's Drive
Columbus, OH 43205
(614) 722-5321
gwenger@chi.osu.edu

Jason White
University of Pittsburgh Cancer Inst.
130 DeSoto St, 315 Parran Hall
Pittsburgh, PA 15261
(412) 624-5358
jason.white@mail.hgen.pitt.edu

Judith Wieland
The Genetics Center
48 Route 25A, Suite 205
Smithtown, NY 11787
(631) 862-3620

John E. Wiley
ECU Brody School of Medicine/
Pediatrics-Genetics
Brody 3E140, 600 Moye Blvd
Greenville, NC 27858
(252) 744-2525
wileyj@mail.ecu.edu

Daynna Wolff
Medical University of South Carolina
165 Ashley Ave, Suite 309
Charleston, SC 29425
(843) 792-3574
wolffd@musc.edu

Shannon Wooton
OHSU Clinical Genetics/Cytogenetics
2525 SW 3rd Ave, Suite 350
Portland, OR 97201
(503) 494-2290
wootons@ohsu.edu

Herman Wyandt, Ph.D
Boston University School of Medicine
700 Albany St, Suite 408
Boston, MA 02118-2526
(617) 638-7083
hwyandt@bu.edu

Kristina Yelavarthi
IUNW Genetics/Cytogenetics Laboratory
3400 Broadway
Gary, IN 46408-1197
(219) 980-6677
kyelavar@iun.edu

Marina Yurovitsky
Carl Ziess MicroImaging, Inc.
One Ziess Drive
Thorwood, NY 10594
(914) 681-7645
myurovitsky@ziess.com

Susan Zneimer
Quest Diagnostics
Cytogenetics Lab
7600 Tyrone Ave
Van Nuys, CA 91405
(818) 376-6096
susan.m.zneimer@questdiagnostics.com